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1952
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ADDRESSES DELIVERED

AT THE CONVENTION OF THE

NATIONAL SHELLFISHERIES ASSOCIATION

Atlantic City, New Jersey

August 12-13-14, 1952

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SHELLFISH SURVEY METHODS

by

Robert L. Dow
Commissioner, Maine Dept. of Sea and Shore Fisheries

A systematic method whereby shellfish populations and volumetric yields may be accurately determined in any shellfish producing area was a problem undertaken by the Maine Department of Sea and Shore Fisheries in 1947.

This investigation was not undertaken for the intrinsic values of the problem itself. With greater emphasis on fisheries management as the most practical approach to the shellfish problem the need for accurate population and productivity information is obvious. This same information would provide for the biologist a basic working background to which other field and laboratory research details might be added. The practicability of a program involving area rotation, open and closed times, period of harvest, frequency of harvest, minimum sizes and other restrictions would be largely dependent upon success in developing accurate population and productivity determinations.

I would like to say we were motivated in our work by these objectives. Unfortunately there were other, more cogent reasons.

In the late summer of 1946 some fifty-eight shellfish areas had been closed in Maine by direction of the U. S. Public Health Service because of sanitary conditions. During the ensuing furor predictions were made that soft shell clam production in Maine, then averaging nine to ten million pounds of meats each year, would immediately be reduced from forty to fifty per cent because of the closures.

Dana Wallace, who was in charge of the Department's shellfish program, first became suspicious of the clam population estimates in specific areas after he casually checked several of these areas with local fisherman and departmental wardens.

It was the result of our suspicions that we began an investigation of this problem. A bacterial resurvey of the fifty-eight areas in 1946 - 47 resulted in the winter opening of several areas. We surveyed these areas and from four were able to obtain accurate production figures to check against the accuracy of our estimates. All surveys made since 1947 have been made in areas where no digging had been carried on for at least one year. Some areas had been closed as conservation areas while other areas did not contain a legal size population.

During the last six years we have surveyed some twenty different areas. Approximately one-half of these areas were not dug commercially because of their small clam population of inaccessibility. We were, therefore, unable to determine the accuracy of our population estimates in those areas.

Altogether we have been able to obtain production figures from the industry on eight separate areas involving ten surveys and ten estimates.

Before discussing survey results, I would like to outline briefly the three principal methods we have used:

1. <u>Compass and chain.</u> The equipment consists of a surveyor's chain or engineer's tape, stakes or rods and a surveyor's hand compass or pocket transit. Sample plots are selected on the basis of a predetermined grid interval. This method controls randomized sampling and reduces human influence in selection to a minimum.

2. <u>Plane table.</u> This method, employing the plane table and telescopic alidade, permits the highest accuracy in area determination. Sample plots are selected by the instrument man to give blanket coverage with no predetermined interval between plots.

3. <u>Photo-enlarged shoreline survey.</u> This consists, in brief, of a photographic copy of a portion of shoreline survey enlarged to convenient size. Convenient size, in this instance, means that orientation of the map on the ground is possible both by inspection and by resection. This size also permits the use of the map reproduction for compiling data as well as for making cartographic corrections.

I have had considerable experience in the use of the engineer sketching set consisting of a small lightweight plane table and tripod, a sight alidade and a clinometer. If no effort is made to include topographic information and only a planimetric survey is desired, the sketching table and sight alidade appears to be the most desirable; since the use of a chain to measure distance between traverse stations would keep to a minimum the normal errors of this method. It permits more rapid work than can be obtained with the plane table and eliminates the extra plotting time in the office made necessary by the compass and chain method.

This year we commenced experiments in the use of vertical aerial photographs. We feel reasonable confident that, by taking our own aerial photographs in conjunction with a plane table ground control, we can establish a reliable and yet comparatively cheap method of completing a topographic survey which would give us a basis for population and productivity estimates as well as topographic changes occurring from time to time.

Total estimates made since 1947 amounted to 38,242 bushels. Total production from these 143.51 acres, ranging in size from 2.2 to 32.8 acreas, amounted to 36,734 bushels or an average estimate error of plus 4.11%. The range of error was from minus 6.0% to plus 26.7%.

Four estimates were made from compass and chain surveys of 29.91 acres. The total volume estimate was 5162 bushels while total production was 4900 bushels or an average error of plus 5.34%. The range of error was from minus 6.0% to plus 26.7%.

Three estimates were made as the result of plane table surveys. Fifteen and four-tenths acres were estimated to contain 24,083 bushels. Production amounted to 23,236 bushels or an average error of plus 3.64%. The range of error was from minus 3.7% to plus 9.0%.

Three estimates made from photo-enlarged shoreline surveys of 81.9 acres amounted to 8997 bushels. Actual production was 8598 bushels or an average error of plus 4.64%. The range of error was from plus .99% to plus 7.0%.

A consideration of estimate error by species does not appear to be valid since the three Venus surveys were made by plane table and the seven Mya estimates were made from compass and chain and shoreline surveys. Venus density averaged 1508.83 bushels per acre while Mya density averaged 120.72 bushels per acre. The range of error for Venus estimates was from minus 3.7% to plus 9.0% while for Mya it was from minus 6.0% to plus 26.7%.

The two basic errors in surveys are errors in area determination and errors in volume determination. The influences which these errors exercise upon the final estimate will be conditioned in part by several factors, all of which can be reasonably controlled.

The type of sediments found in the areas already surveyed appears to have had little or no influence upon the accuracy of the estimate. Since these types are generally characteristic of the sediments found in Maine's shellfish producing areas it is reasonable to conclude that sediments will not affect the accuracy of any future surveys except as they may influence the accuracy of the size of samples taken.

The second most accurate estimate made was plus 1.15% error and the least accurate was plus 26.7%. Both areas consisted of silt-sand-pebble sediment mixtures.

The notable exception in which sediment types can substantially influence the accuracy of area determination is the case in which flocculent unstable sediments do not permit the establishment of plane table or sketching table survey stations within the area.

The size of the intertidal areas surveyed appears to have had some influence on the accuracy of our estimates.

The four estimates made on the three largest areas had errors of plus 7.0% or less. Two of the three smallest areas had errors of plus 15.2% and plus 26.7%, the largest experienced. It is likely that the size-density ratio of an area, local physical-topographic conditions and the size of the sampling fraction are the factors influencing the degree of error estimate.

The two major errors of plus 15.2% and plus 26.7% were experienced in two small areas where the intertidal strip is narrow and broken by ledge outcrops. The 26.7% error estimate was based on the smallest sampling fraction, 1/17,787, of any survey made. In order that representative sampling of an area may be attained, it seems desirable to make the sampling fraction at least 1/6,000. The average for all our surveys was 1/5,764 while the range was from 1/1,498 to the above 1/17,787.

Since that intangible factor, digging intensity, varies in Maine seasonally, geographically, according to competition from other fisheries and industries and is appreciably influenced by marked conditions and other factors beyond imagination, we abandoned our initial proposal to limit our estimate to the probable commercial production of the area concerned. We have defined our estimate as the probable population occupying the area bounded by the limits of the survey.

The results obtained thus far indicate that our methods are sound and offer considerable promise. A maximum tolerable error of plus or minus 10% appears to be possible. Eight of the ten estimates we have been able

to check for accuracy have ranged between minus 6.0% and plus 9.0% or a net error range of 15.0%. Those estimates accounted for 87.31 acres or 95.83% of the total acreage surveyed. These same estimates accounted for 35,134 bushels or 95.64% of the total production.

Two estimates were made from one survey in Scarboro. Since the survey was made after the growing season had commenced we used known growth rates in the area to project one estimate through an additional year of growth. The second estimate was based on the probable volumetric yield during the remainder of the then current growing season. A$_t$ the end of the full year, two fishermen were still averaging one-half bushel of clams per day apiece.

Two surveys, one resurvey and a random check of mortalities in two Brunswick hard-shell clam areas resulted in projected volume estimates. The resurvey and the random check had served to establish that mortalities of 40.3% and 30.0% had occurred in the two areas the preceding winter. Using the known growth rates for the two areas during the previous growing season, the volume increment for the survivors was projected through the following growing season. It was anticipated that certain errors would exist in this estimate, although only one allowance was made for them, since commercial digging operations were being carried on throughout the growing season. The only correction made was one of 6.0% by volume based on the hopeful assumption that the average fisherman would not violate the 10% by count tolerance of clams less than two inches in diameter.

The percentage of surviving animals determined from the resurvey and random check was used to estimate the probable percentage of clams produced in each area. Cumulative production for the combined areas was arbitrarily apportioned between the two areas on the basis of the projected estimates. The error in estimate was plus 9.0% for one and plus 4.7% for the other. As there was still a residual population in each area, the true error of the estimates rapidly approaches zero.

I would like to comment briefly on the last survey we have made. This was in Wells, an area which had been closed for conservation purposes and one which we had been using as an experimental management area. The survey and estimate was made just prior to the opening of the area for digging. The area was reclosed at the end of the winter digging season with a considerable population of clams remaining in the flats. A resurvey of the area was made, using the same method employed in the original survey. The residual population was estimated to be 548 bushels. It was, therefore, determined that the original estimate error was plus .99%.

Although the total number of areas surveyed is not sufficient to establish conclusively the relative accuracy of the various methods employed, I am of the opinion, formulated on the basis of experience and the results obtained thus far, that certain critical observations can be made.

Photo-enlarged shoreline surveys permit low cost rapid reconnaissance-surveys which have been surprisingly accurate. Particular attention must be given to mapping errors in order that corrections may be made. This method is especially useful in surveying tortuous tidal river areas where the use of either the plane table, sketching table or compass and chain would be slow and expensive.

The compass and chain method of survey is perhaps best suited to long and narrow tidal areas where population densities are not high or drastically variable and the acreage to be surveyed is considerable. I would not recommend this method in an irregularly shaped area or one broken up by topographic features.

In small or irregularly outlined areas or in areas where high population concentrations require meticulous work, the plane table is unexcelled. For accurate areal determination, the plane table is a necessity. The high field cost of this method is, to a great extent, offset by the short period of office time required to compile the final estimate.

Obviously the accuracy of any survey method outlined here or otherwise developed is governed by the skill of the manipulator. For one lacking experience and skill in the manipulation of the telescopic alidade, or even in the sketching table, the compass and chain method is the most practical, particularly if stakes or rods are used for control purposes in establishing base lines and in turning angles.

The selection of method to be used should be made only after a reconnaissance of the area to be surveyed has been made. The plane table, the sketching table, the compass and chain and the photo-enlarged shoreline survey all have their limitations. Reconnaissance of the area to be surveyed will permit the selection of the method which will give the most accurate results for that particular area.

We realized in setting up our investigation that we had no backlog of conversion tables, estimate adjustments and other information necessary to the development of the most accurate results. The refinement and modification of methods herein described will be a constant operation taking place as additional information is acquired.

METHOD OF SURVEY

Instruments	Compass & Chain	Plane Table	Photo Enlarged Shoreline Survey
Number of estimates	4	3	3
Total acreage	29.91	15.4	81.9
Average acreage	7.48	5.1	27.3
Total estimate	5,162	24,083	8,997
Total production	4,900	23,236	8,598
Aver. vol. per acre			
Estimated	172.58	1563.83	109.85
Produced	163.82	1508.83	104.98
Average error	≠ 5.34%	≠ 3.64%	≠ 4.64%
Range of error	-6 to ≠26.7%	-3.7 to ≠9.0%	≠.99 to ≠7.0%
Net range	32.7%	12.7%	6.01%

SURVEY BY SPECIES

	VENUS	MYA
Number of estimates	3	7
Acres	15.4	111.81
Estimate in bushels	24,083	14,159
Production in bushels	23,236	13,498
Range of error	-3.7 % to ≠9.0 %	-6 % to ≠26.7 %
Net Range	12.7 %	32.7 %
Average error	≠ 3.64 %	≠ 4.89 %
Aver. est. volume per acre	1563.83	126.63
Aver. production per acre	1508.83	120.72

MAINE SHELLFISH SURVEYS

Place	Date	Sediments	Instruments Used	Area In Acres	Estimate in Bushels	Production in Bushels	Production per Acre	S/F	Species	Error of Estimate
Harpswell	Nov. '47	Silt - sand cobble	Compass & chain	15.8	1410	1500	94.93	1/11283	Mya	-6.0 %
Hulls Cove - Salisbury Cove	Dec. '47	Silt - sand pebble	Compass & chain	7.01	2023	2000	285.31	1/2994	Mya	+1.15%
Jonesport	Dec. '47	Silt - sand- cobble-boulder	Compass & chain	2.2	462	400	181.81	1/1498	Mya	+15.2 %
Jonesport	Dec. '47	Silt - sand- pebble	Compass & chain	4.9	1267	1000	204.08	1/17787	Mya	+26.7 %
Scarboro	May '50	Silt - sand	Photo-enlarged shoreline survey	32.8	3216-7mos. 3765-12 "	3000 3600	91.46 109.76	1/7212 1/7212	Mya Mya	+7.0 % +4.58%
Brunswick	Oct. '50	Sand - clay silt	Plane Table	3.3	6637	6887*	2086.97	1/4080	Venus	-3.7 %
Brunswick	Nov. '50	Sand - clay silt	Plane table	5.1	8253	7568**	1483.92	1/4756	Venus	+9.0 %
Brunswick	Apr. '51	Sand - clay silt	Plane table	7.0	9193	8781**	1254.43	1/2895	Venus	+4.7 %
Wells	Sept. '51	Silt - sand	Photo-enlarged shoreline survey	16.3	2016	1450 548*	122.58	1/1890 1/1793	Mya	+ .99%
			TOTAL	110.71	38242	36734				
			AVERAGE	11.07	3824	3673		1/5764		+4.11%

* Resurvey

** Estimate projected on basis of resurveys and known volume increment in these areas.
Only total production figures were available.

Number of separate areas surveyed: 8 - 91.11 acres
Number of surveys: 10 - 110.71 acres
Number of estimates: 10 - 143.51 acres

A CULTURE TECHNIQUE FOR THE DIAGNOSIS OF INFECTION WITH DERMOCYSTIDIUM MARINUM IN OYSTERS[1]

Sammy M. Ray[2]

August 1, 1952

INTRODUCTION

Briefly, the culture technique for the diagnosis of Dermocystidium marinum Mackin, Owen, and Collier, 1950, in oysters consists of two principal steps: (1) planting of oyster tissue in a suitable nutrient medium fortified with antibiotics to suppress bacterial growthe, and (2) microscopic examination of tissue for parasites, which become conspicuously enlarged into cyst-like bodies and can be rendered easily recognizable by a characteristic blue reaction to iodine.

MATERIALS AND METHODS

A satisfactory nutrient is fluid thioglycollate medium (Difco) rehydrated with sea water, if available, or distilled water containing 20 grams of NaCl per liter and dispensed in tubes in 10 ml. amounts. The screw-cap culture tube of 125 x 16 mm. is a convenient size, and the screw caps not only make it feasible to carry the tubes into the field, but also in the laboratory they reduce the speed with which the medium absorbs oxygen. The anaerobiosis of the medium suppresses the growth of molds and Protozoa but is not essential for the enlargements of the parasites. Thioglycollate media which contains methylene blue as an oxygen indicator should not be used since the blue color of this dye in its oxidized state might possibly mask the diagnostic color reaction of the parasites when treated with iodine.

Streptomycin and penicillin solutions are added to each tube of medium after sterilization to give a concentration of 250 units of each per ml. of medium. When badly decomposed tissues of unusally large amounts of tissue are used, the concentration of antibiotics should be increased. Concentrations up to 1,000 units of each antibiotic per ml. have been successfully employed. The antibiotics and medium should be thoroughly mixed by vigorously swirling the medium in the tube. Tubes containing antibiotics may be stored at room temperature for several days; however, there is a noticeable loss in antibiotic potency and they

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should be refortified with antibiotics if not used within a week. Complete sterility is not necessary although retardation of heavy bacterial growth during early period of incubation is necessary for the enlargement of the parasites. The early development of heavy bacterial growth in cultures results in the failure of the parasites to enlarge normally; nevertheless they become blue when treated with iodine.

Vials containing 200,000 units of crystalline penicillin G (sodium or potassium salt) are obtained locally at a cost of 40 cents each, and vials containing 1 gram of crystalline dihydrostreptomycin sulfate (1 gram of streptomycin is equivalent to 1 million units) are obtained at a cost of 85 cents per gram. The procedure used by the writer for preparing antibiotic solutions is given below: By adding 7 mls. of sterile distilled water to a vial containing 200,000 units of penicillin G and 5 mls. of sterile distilled water to a vial containing 1 gram of dihydrostreptomycin sulfate, solutions are obtained from which a stock solution containing 25,000 units of each antibiotic per ml. can be prepared by adding aseptically 1 ml. of the streptomycin solution to a vial containing 7 mls. of penicillin solution. One tenth of a ml. of this stock solution added to each tube of sterile medium by means of a sterile tuberculin syringe (1 ml.) will give a concentration of approximately 250 units of each antibiotic per ml. of medium. All antibiotic solutions should be frozen to prevent appreciable loss of potency. When antibiotics are purchased at the prices listed above, the cost of each tube of medium (10 ml. amount) fortified with antibiotics is slightly more than one cent. The antibiotics usually may be purchased more economically from wholesale drug companies for research purposes.

D. marinum has been cultured in the following tissues of dead and living oysters: Gill, palp, mantle, heart, adductor muscle, rectum, and also small entire oysters. Routinely the writer incubates the heart, rectum, and pieces of gill and mantle (about 5 x 10 mm.) from each oyster. These four tissues should be cultured if an extremely thorough diagnosis of light infections is desired. For survey work where large numbers of oysters are to be examined, the incubation of the rectum will suffice for a diagnosis. D. marinum with rare exceptions has been found in this tissue whenever it has been found in any of the other tissues routinely employed. Occasionally a few parasites have been detected in rectal tissue when none were observed in the other tissues. Usually the parasites have been found to be more abundant in the rectum than in other tissues in case of light or moderate infections. In heavy infections all tissues generally contain the parasites in approximately equal abundance. The transparency of the heart enables one to observe the parasites with comparative ease. In rare instances a few parasites have been observed in mantle tissue when none were observed in the heart or other tissues. Likewise, with even greater infrequency, a few parasites have been observed in gill tissue alone. Sometimes there is a marked difference in the concentration of the parasites in different tissues and even in different areas of the same tissue. This has been observed more often in living oysters than in dead or gaping ones.

When tissues are excised from a live oyster within a few hours after removal from water, the four tissues routinely cultured may be placed in the same tube. Tissues from gaping or recently dead oysters may be treated in the same manner. However, oysters that have remained

closed for 24 hours or more, even when iced, build up a very heavy, or at least very resistant, bacterial load. There have been frequent failures in attempts to retard this bacterial load even by considerably increasing the concentration of antibiotics in addition to placing each tissue, if more than one were cultured, in a separate tube. In order to reduce the bacterial load in such closed oysters so that it may be retarded by the usual concentration of antibiotics, it is advisable to place the oysters in circulating sea water (aerated, noncirculating sea water has proved satisfactory when circulating sea water is not available) for at least 24 hours before excising the tissues for incubation. To minimize the concern with increased bacterial load that may arise from prolonged removal of oysters from water during transit from the field to the laboratory for diagnosis, the writer has found it advantageous to carry into the field tubes of medium previously fortified with antibiotics using 500 units of each antibiotic per ml. of medium if the tubes are not to be used within 3 or 4 days during warm weather. This procedure enables one to excise and plant the tissues in the field within a short time after the oysters have been removed from the water.

A minimum period of 48 hours of incubation at room temperature (18° C to 30° C.) in a closed, dark cabinet is recommended. The average size of unenlarged D. Marinum in oyster tissue is approximately 10 μ. The organisms in cultured tissue begin to enlarge in about 10 to 12 hours but do not begin to react to iodine and are not easily detected until after approximately 18 hours. At this time a few have reached a diameter of about 35 μ though the majority are considerably smaller; their walls are thin and stain a rather faint blue with iodine, and, since the cytoplasm stains a yellowish brown, they have a greenish appearance. As the parasites continue to grow, the wall thickens and stains a deeper blue and the single vacuole enlarges. With increasing age the iodine reaction becomes more immediate and more intense until, in cultures one to several weeks old, the parasites appear as opaque, blue-black bodies. Meanwhile, the cytoplasm becomes more and more reduced as the vacuole becomes greatly enlarged, usually with the accumulation of fat droplets. The size gradually increases in a maximum of 40 μ to 45 μ at 24 hours, 70 μ at 48 hours, and 90 μ at 72 hours. In cultures a week to several months old some parasites reach a diameter of 100 μ to 150 μ. In these old parasites the cell wall has a thickness varying from 1.5 μ to 16 μ. In a single oyster from South Carolina the majority of the cultured organisms exceeded 200 μ, some attaining 280 μ. A few parasites reaching a diameter of 200 μ have been observed in infected oysters from Biloxi, Mississippi. An incubation period of 24 to 36 hours is required for a strong blue reaction with iodine regardless of size of the organism. Parasites after growth show no evidence of disintegration after prolonged incubation even when the medium is contaminated with bacteria.

For examination the tissues are placed on slides and examined under cover slips at about 100 X magnification. If numerous and well-developed the parasites are conspicuous without staining. However, best results for rapid and accurate diagnosis are obtained by treating the tissues with iodine solution[1] before examination. This treatment is especially helpful in the

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[1]The concentration of iodine solution recommended is a 1:5 aqueous dilution of Lugol's stock iodine solution prepared according to the following formula:

                    Iodine— - - - - - - - - - - - - 5 gms.
                    Potassium iodide - - - - - - - - 10 gms.
                    Distilled water - - - - - - - -100 mls.
Mix I_2 and KI by grinding in a mortar; add water in small amounts until I_2

parasites are scanty or small because they are more easily seen and more readily differentiated from oil globules, diatoms, oyster eggs, and other spherical bodies of doubtful nature that are frequently encountered in rectal and adjacent tissues. The best procedure is to shred the tissue well with teasing needles to allow for greater penetration of iodine as well as to allow many of the cyst-like bodies, if present, to fall free. The teased tissue is flooded with several drops of iodine solutions and allowed to stand for approximately 5 minutes before covering the preparation with a cover slip. The excess solution should be removed with strips of absorbent toweling or filter paper while flattening the tissue as much as possible by pressure on the cover slip. The tissues absorb iodine very readily and it may be necessary to restain the preparation if the amount of tissue is large. Some fragments of the tissue should be stained brown at least at the edges.

In heavily infected tissues so treated the blue color of the parasites is easily seen with the naked eye. In stained preparations the majority of the bodies appear in varying shades of blue; some appear green, greenish-brown, and brown. The blue color is restricted to the wall of the parasites and the varying shades of blue, green, and brown depend on the thickness of the wall and amount of cytoplasm present as well as on the concentration of iodine in various areas of the preparation. The blue color appears almost immediately upon addition of iodine but it may begin to lose its intensity in a few hours; however, the bodies may be stained repeatedly. Enzymatic and other tests indicate that the blue color is not due to starch; possibly is due to some glucoside, such as saponarin in the wall. In heavily infected tissues the total volume of the pieces is greatly increased, and the original tissue is practically obliterated by the mass of cyst-like bodies. This results in many of the bodies becoming free in the tubes or on the slides to which the pieces are removed.

The blue color demonstrated by cultured D. marinum after treatment with iodine is sufficient to distinguish this parasite from practically all other spherical objects that the writer has encountered in incubated oyster tissues. However, on rare occasions a very few unidentified spherical or oval bodies within the size range of cultured D. marinum have been observed. These bodies appear as purple or blue-black, opaque objects, even in very dilute concentrations of iodine. When only a very few purple or blue, opaque bodies are observed, the observation of a distinct vacuolation of the cytoplasm in some individuals as well as the blue color is necessary for the accurate diagnosis of D. marinum. Although as previously stated, the majority of the parasites in cultures a week or more old appear as opaque, blue-black bodies when treated with iodine, usually many of them showing a distinct vacuolation of the cytoplasm may be found in areas of lesser iodine concentrations. Infrequently cysts of Protozoa have been observed in old cultures. These cysts might possibly be mistaken for small D. marinum, but the dense cytoplasm possessed by such protozoan cysts so far observed enables one to differentiate them from cultured D. marinum. Some of the protozoan cysts show a light purple color when treated with iodine.

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1 (cont. from page 11)
   and KI are dissolved; allow to stand 24 hours; then filter and store in
   glass stoppered bottle. Due to gradual deterioration of the diluted iodine
   solution, it should be prepared anew at approximately monthly intervals.

DISCUSSION

The presence of D. marinum has been demonstrated by the culture technique in oysters from Pensacola, Florida; Wadmalaw River, South Carolina; Biloxi, Mississippi; and numerous areas in Louisiana. The method has these advantages over the histological techinques usually employed: First, economy of time and labor; second, diminution of equipment and skill required for a reliable diagnosis; and third, increased accuracy in the detection of light infections. Since dead and badly decomposed tissues usually make unsatisfactory histological preparations, this technique has the added advantage that it permits diagnosis of D. marinum in dead or decomposing oysters. At present, it is uncertain whether the number of cells increased during incubation although the evidence thus far obtained seems to indicate that multiplication of the enlarged forms rarely occurs in thioglycollate medium. Tissues containing a few parasites have been examined after varying periods of incubation ranging from 48 hours to several months without indication that the organisms increased in number. Multiplication, however, prior to enlargement might possibly occur. Presently, therefore, it is the opinion of the writer that, with rare exceptions, the technique may be used to indicate intensity of infection as well as incidence.

In some media the enlarged parasites bud, producing hyphae and some increase in the number of individuals, but this has been observed in only four cultures out of several hundred where fluid thioglycollate medium has been utilized as nutrient. In the four cultures referred to above, the period of incubation ranged from 23 days to 7 months. The buds and hyphae give a blue reaction with iodine although the walls are thin. In the cultures where hyphal and budding forms have been observed, the cytoplasm of many of the cyst-like bodies is granular and non-vacuolated. This condition of the cytoplasm has not been observed in the bodies normally developing in fluid thioglycollate medium. The production of hyphae and budding forms definitely establishes D. marinum as a fungus.

This technique should prove to be of great practical importance for rapidly, accurately, and economically determining D. marinum incidence in outbreaks of oyster mortality, and it should greatly facilitate studies of the distribution of the parasite. It has also proved to be a valuable aid in experimental studies of the life cycle and biology of the organism.

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EFFECT OF HEAVY INFESTATIONS OF POLYDORA WEBSTERI HARTMAN ON
CRASSOSTREA VIRGINICA (GMELIN) IN LOUISIANA

By
J. G. Mackin[1] and Fred F. Cauthron[2]

[1]Texas A.&M. Research Foundation and Department of Oceanography, Agricultural and Mechanical College of Texas, College Station, Texas.
[2]Texas A.&M. Research Foundation, Grand Isle, Louisiana.

HISTORICAL

Many observers have studied the effects of infestation of oysters by various species of the genus Polydora Bosc. Only the most important papers will be discussed here and no attempt has been made to complete a bibliographical list. The discussion is divided into analyses of researches in three regions: (1) Australia, (2) the coast of Europe, and (3) the east and Gulf coasts of North America.

Some of the earliest studies on Polydora were made in Australia. Most frequently referred to is the paper by Whitelegge (1890). This author investigated mortalities of oysters in the Hunter River estuary and concluded that Polydora ciliata was directly responsible for deaths of large numbers of oysters, and indicated that he believed that widespread mortalities in other areas of Australia were caused by Polydora. He attributed the lethal effect to (1) accumulations of putrescent mud in thinly covered blisters, (2) imperfect closure of valves due to mechanical and chemical damage, (3) lessened food supply of the oyster, due to competition for food on the part of the worms. He considered that putrescent mud was the most important effect.

Whitelegge's conclusions were evidently widely accepted in Australia. Roughley (1925) says, "The greatest enemy the oyster has on the coast of New South Wales is the mudworm (polydora ciliata). So great an influence has been exercised by this worm that it has altered the whole type of cultivation practised".

However, McIntosh (1902) did not agree, and critized Whitelegge's report. Kesteren (1941) states that the role of the mudworm in destruction of oysters is not clear but pointed out that measures taken to eliminate or reduce the degree of infestation of oysters by Polydora were successful in rehabilitation of the industry, certainly a strong argument in favor of Whitelegge's conclusions. The better survival in oysters with small infestation as compared with low survival in oysters with heavy infestation is in one sense a rough experiment on a huge scale. It is inconclusive however, in that (1) the physical environments of the "experimental" and "control" groups were not comparable, the former being in deep water, the latter on the foreshores, and (2) no attempt was made to determine whether or not the two groups were equal as regards infection with disease of protozoal, fungal or bacterial origin.

It is possible that the species of Polydora infesting Australian oysters is not P. ciliata as thought by Whitelegge. Korringa (1951) believes that the Australian species is P. hoplura or a species with kindred boring habits, since he says

that the latter is injurious to oysters, while P. ciliata produces relatively light damage.

A number of European workers have observed damage to oyster shell by species of Polydora. Apparently P. ciliata and P. hoplura occur throughout the northeastern Atlantic, living and burrowing in oyster shell. These species have been confused with each other and with other species (Korringa, 1951). Giard (1881) regarded Polydora as an enemy of oyster culturists. Carazzi (1893) claimed that oysters were destroyed by Polydora. He observed that the worms often bored into the adductor muscle and thereby destroyed the insertions of the fibers, causing the oyster to gape. Hoek (1902) also believed that Polydora could destroy oysters when the infestation was heavy, and when the worms drilled through the shell.

Dolfuss (1922) reviews the work of various workers up to this time who studied the Polydora problem and listed effects of the worms, most important of which were (1) the introduction of bacteria and development of hydrogen sulphide in the mud blisters, (2) chambering of the shell by repeated coverings of nacre over mud blisters, (3) stunting of growth of oysters, (4) riddling of shells to make them fragile (5) adverse effects on condition. Dolfuss believes that P. hoplura produces more damage than does P. ciliata.

Leloup (1937) gave a detailed account of the destruction of planted oysters in the basin of Ostend (France). According to this author the worms were extremely numerous in the mud of the bottom and attacked the planted oysters in great numbers. They so riddled the margins of the right valve that malformation of the left valve resulted. The oysters were unable to effect efficient closure of the shell and died in large numbers (60 per cent). The author also stated that the destruction of the shell margin made the oysters less resistant to attacks of crabs (Carcinus moenas).

Lamy and Andre (1937) studied annelids which perforate the shell of oysters and gave a detailed account of researches on Polydora. These authors think that if infestation with Polydora is heavy, the oyster will die from the effects of fatigue induced by the necessity for abnormal secretion of shell materials. They cited no specific observations of their own in support of their opinion.

Korringa (1951) differentiates between damage by P. ciliata, which he considers generally small, and P. hoplura, which he considers to be more extensive. He says that the latter is not a true Dutch species, but belongs to the coast of France, but cites the year 1950 as one in which the species, introduced with imported oysters, reproduced in Holland. The chief damage, according to Korringa, lies in stunting of growth and decreased weight of heavily infested oysters and he cites figures to support his opinion. Other lesser effects are also claimed. The same author (1951a says that P. ciliata probably does not occur in America or Australia and attributes most cases of severe damage and high mortality to P. hoplura or P. websteri. He intimates (but does not say) that weak oysters in subtropic climate in summer may die because of inability to seal off Polydora penetrations in the shell.

On our own side of the Atlantic, Lunz (1938, 1940, 1941) initiated studies on the effects of Polydora on Crassostrea virginica. He showed that the percentage of infestation depends on the type of bottom; the muddier the bottom, the more worms in the oysters. More to the point, an analysis of oysters showed that there was a lowering of fitness, according to the Medcof formula, in those oysters more heavily

fested. Medcof later (1945) in a similar study of Canadian oyster beds, found no such correlation to exist but found that Polydora blisters detracted from the market value. It may be remarked concerning Medcof's work that Canadian oysters, according to his figures, do not have infestations of any significance. Three worms (or blisters?) per oyster was the heaviest average concentration recorded. Where Polydora is a problem, such low infestation would be counted as zero.

Nelson and Stauber (1940) attributed the loss of several hundred thousands of dollars worth of oysters in Delaware Bay to infestation with Polydora ligni. Mud deposited on the surface of shells by the worms decomposed, liberating hydrogen sulphide which presumably created a high oxygen demand thus smothering the oysters and Polydora alike.

Kavanaugh (1940) described in considerable detail the effects of Polydora on the Japanese oyster, Crassostrea gigas. The oysters had been imported to Louisiana for experimental purposes, the intention being to develop hybrids with the native oysters, Crassostrea virginica. However, the Japanese oysters, after arriving in good condition, sickened and some died after four to eight weeks. They dropped in "meat content" about 88 per cent. Polydora blisters formed thickly inside the shell. In a weakened condition some survived for several months. Although the author does not say so, it is presumed that none survived. He tentatively attributed the condition of the meats and the deaths to Polydora ciliata, but stated that "no one could afford to state positively" that it was so. Nevertheless it was obvious that he was convinced that Polydora was the cause. Peculiarly he seemed to think that Polydora did not attack the native Crassostrea virginica.

Loosanoff and Engle (1943) compared infestations of Polydora in oysters in trays above the bottom and in oysters on the bottom. They found (1) that infestations were heavier in the tray oysters, (2) tray oysters were in better condition and had less mortality than those on the bottom.

Grice (1951), working at the Bears Bluff Laboratories in South Carolina, studied incidence of Polydora in various size groups, and seasonal variation in incidence. He postpones the problem of etiology of lethality to future studies, stating that there is no evidence that high mortality in subtidal oysters is caused by Polydora.

POLYDORA IN LOUISIANA

In this paper it is proposed to examine some of the data having to do with (1) the association of Polydora with abscess and ulcerations, (2) shell damage, especially to the thin growing margins and (3) the problem of lethality. In that part of the Louisiana oyster-producing territory lying between the Mississippi River and Bayou La Fourche, infestations with Polydora websteri are probably heavier than have ever been recorded heretofore (Fig. 1). The number often exceeds 500 worms per oyster; occasionally it is more than 1000 per oyster, and in rare instances cases of more than 2000 have been recorded. The studies therefore required special methods which have been developed as the work progressed. The number of shell blisters is not an indication of number of Polydora in the shell. In order to be able to procure statistical data on the number of Polydora in the shells of oysters, a considerable amount of time was devoted to development of a method of extraction, so that

worms could be counted. Without detailing the various methods studied, one was found which was satisfactory and statistics given are based on that one. It consists of treatment of oysters with a solution of phenol in sea water approximately in a ratio of 1 to 2000. Worms are not killed by this solution for as much as two days but are so irritated that the continued muscular contractions cause them to leave the burrows and fall to the bottom of the container. Small numbers of oysters were treated in finger bowls or in battery jars; large numbers of oysters, packed in trays, were dewormed in a large iron vat. When worms were extracted for purposes of counting, the oysters were thoroughly scrubbed to remove debris and worm tubes, before the phenol solution was applied. The worms collected in the bottom of the jar were then relatively free of debris. Large numbers of worms were measured for volume in a 100 cc graduate, a sample of 1 cc counted, and the total number of worms computed. Worms are reported as number per oyster. Fig. 2 shows an oyster being treated for extraction of Polydora and the appearance of the worms as they emerge.

Polydora and Ulcerations.

In high temperature months many oysters in Louisiana develop yellow-colored ulcerated areas on the viscera and especially in the adductor muscle (Figs. 3, 4, and 5). Carazzi (1893) in Italy and Whitelegge (1890) in Australia found these ulcerations to be associated with Polydora infestation. They are not always so, for some are obviously assoicated with either Cliona penetrations or with Martesia, and a large number may not be directly associated with any one of the three common shell pests. Figs. 6, 7, and 8 illustrate the type of ulceration and the obvious association with penetration of the internal nacre of the shell by Polydora. When Polydora bores into the adductor muscle from the shell burrow a small mud vesicle is forced into the tissue of the muscle by the worm. This causes an abscess to form and the surrounding muscle fibers to disintegrate. If the disintegrated area is large enough, the oyster gapes and death ensues. In most cases only a small area of destruction results from penetration. In such cases the oyster phagocytizes the necrotic tissue, develops a secreting epithelial layer around the mud vesicle and deposits first conchyolin, then limy and nacreous shelly materials around it. These occluded mud vesicles are very common and are the familiar "muscle perrls" of Gulf coast oysters. Most authors are apparently of the opinion that an oyster cannot secrete limy materials around foreign objects within the adductor muscle. However, extensive histological and experimental study has shown that it is a normal process of recovery, but is delayed considerably over the period required to seal off a penetration of the shell in any area outside of the muscle attachment. This delay may result in the death of the oyster. If the latter is in any way subnormal, repair is slow, and saprophytic bacterial toxins may so enlarge the area of muscle necrosis as to involve approximately all of it. Such cases result in gaping and death. Figs. 9 and 10 show the entry point of a Polydora burrow and the small mud vesicle, around which are concentric rings of necrosis. The oyster and the mass of yellow pus and debris were removed to allow photographing of the point of entry. The few straggly muscle strands were all that remained of the original adductor muscle.

Results of a study of 290 oysters from five stations in the Barataria Bay area give some idea of the incidence of ulcerations (Table 1). The survey was conducted in October and the oysters had been kept at their respective stations for more than a year. Oysters which have not passed through the summer period in the high salinity waters rarely develop ulcerations, and oysters under one year of age are less likely to become ulcerated than are those in the second and third years. Table 1 shows

t. t 27 per cent of the 290 oysters examined had ulcerations. About one third of these were obviously induced by contact with mud introduced into the shell cavity by Polydora. Most of the Polydora-induced ulcerations were in the adductor muscle.

It may be pointed out that most investigations of mortality of oysters have encountered high percentages of ulcerated and abscessed oysters in the endemic area. Thus high percentages of ulcerations and high mortalities are generally associated. It remains to be proved that ulcerations are a cause of death on a wholesale basis, although small incidence of mortality can be demonstrated to result therefrom. It is much more likely that most ulcerations are a result of poor condition caused by disease than that they are themselves a primary cause.

TABLE 1

ULCERATIONS OF OYSTERS AND RELATION TO POLYDORA, MARTESIA AND CLIONA

Barataria Bay Area, Louisiana, October, 1949

Station	Oysters Examined	Number of Ulcerations	Ulcerations Associated With							
			Polydora		Martesia		Cliona		Undetermined	
			No.	%	No.	%	No.	%	No.	%
Chene Fleur	60	7	1	14					6	85.7
Bassa Bassa	60	10	3	30	1	10			6	60.0
Sugar House	60	32	8	25	13	40			11	34.4
Bayou Rigaud	50	16	9	56	1	6	2	12	4	25.0
Grande Ecaille	60	13	6	46					7	53.9
Totals	290	78	27	35	15	19	2	3	34	43.6

Shell Damage by Polydora.

Extensive damage to the shells of oysters has been reported by various authors. While most of these considered blistering of the internal surface to be the most important damage, a few observed that large numbers of worms could destroy the shell margin of the right valve, thereby inducing a misfit of the valves and consequent poor closure. Heavy mortality has been attributed to this imperfect closure.

It was noted early in the studies on Polydora that heavy infestations of the shell margin often resulted in apparent shortening of the shell and were sometimes accompanied by heavy mortality. A study was set up designed to measure the extent of destruction of the shell margin and data were taken on accompanying mortality. It should be noted that the fact that mortality occurred as the shell margins were destroyed does not mean that the evidence shows that the one resulted from the other. This study is in no sense an experiment. It is a measurement of shell destruction directly observed to be the work of Polydora.

Thirty-six oysters were placed in numbered compartments of two trays. Oysters used were heavily infested with Polydora. The trays were placed in a small bayou on a natural oyster bed where heavy Polydora damage has been observed. Tidal current was swift, and the trays were held in place by lashing them to poles forced into the shell bank. The right valve of the oysters was measured at the beginning of the study in the latter part of May, again near the end of July, a third time in early October and the last time in the middle of November. Some oysters were found dead at the first inspection after the initiation of the study. These were not included in the tabulations of results. Sixty-seven per cent died at some time during the May to November period. A surprising loss of shell was shown by the measurements. Those remaining alive to the end of the study were the best index of destruction. These lost an average of six mm in length or approximately six per cent during the July to October period. All others lost an average of about 9.5 mm in shell length in the same period. Slight average losses occurred both before and after the mid-summer retrogression. The data are summarized in Fig. 11. Mortality rate was highest during the period of greatest destruction of shell.

At termination of the study all surviving oysters were treated with phenol and the resulting extruded worms were counted. The oysters averaged more than 1200 worms each. Fig. 12 shows the process of worm extraction in some of the oysters.

Polydora and Oyster Mortality.

A number of attempts were made to measure the extent to which Polydora websteri may destroy oysters. All attempts were inconclusive for one reason or other. This section describes one of the experiments, and the results obtained. In addition there is a discussion of the factors which made the attempt inconclusive.

Since it was possible, by means of phenol treatment, to rid oysters of Polydora, it was considered feasible to use such dewormed oysters as controls in experiments designed to measure lethality. Accordingly approximately 800 oysters from the Chene Fleur Station were dewormed with phenol and placed in trays. Another group of about 800 wormy oysters from lower Barataria Bay were used for the experimental group. Controls and experimental oysters were placed in alternate positions (trays) in a small bayou which contained natural reefs of wormy oysters. This bayou was chosen because the conditions there would maintain the infestation in experimental oysters. Cliona, Martesia, and Thais, which are possible complicating factors, were absent from the location. The presence of small natural reefs of oysters showed that the area was more suitable for oyster growth than is lower Barataria Bay, where natural reefs have long since disappeared, but in which nevertheless oyster culture is practised.

Control and experimental oysters were placed on a specially prepared rack and mortality checks were made periodically for six months. Control oysters were de-wormed at the beginning of the study on July 9-10, and again on August 19. Periodic counts showed that the control oysters were only relatively free of worms, that sub-sequent to deworming they accumulated up to 100 worms per oyster. However, the experimental oysters always had about 400 to 600 worms per oyster. Controls grew rapidly throughout the period of the study, while the experimental oysters had the growing edge destroyed as fast as it was produced up to about the end of September. Thereafter experimental oysters also added to the shell length.

Table 2 and Fig. 12 summarize the data. It will be seen that during the warm months mortality in the experimental group was two to six times the rate in the con-trol group. From October to January the mortality was for practical purposes the same, so that the difference in mortality for the entire period was actually pro-duced in the three months period of July, August, and September. Total mortality for the experimental group was 46 per cent; for the controls it was 27 per cent.

This difference is statistically significant. However, one feature of the pat-tern of mortality was peculiar. The mortality rate in the experimental oysters was highest at the beginning of the study and steadily declined from July to the end of the following November. A decline during cool months would be expected but decline in warm months would occur only if the new environment were relatively free of some lethal factor belonging to the environment from which the oysters were taken origin-ally. Whatever the factor was, it could be transported with the oysters. Only a disease could be thus transported. Subsequent investigation showed that the Chene Fleur oysters were in fact relatively free of Dermocystidium disease and the lower Barataria Bay oysters had a high incidence of the fungus. Thus there were at lease two variables, and it is not possible to tell from the data just what part of the mortality in controls and experimental oysters was caused by either one.

One other feature of the study is very interesting, although it has little to do with Polydora. The location of the experiment was in an area with very high tem-peratures in summer and very considerable fluctuation. Water temperatures have been measured at 37° to 40° C and unquestionably attained that height almost daily during the summer. Exceptional days would be those which were cloudy.

But the data showed that the Chene Fleur oysters grew very rapidly throughout the hot period, many adding more than an inch in length. Checks showed that the meats were in excellent condition at the end of the summer, although they were rated at only fair at the beginning. These oysters were not "acclimated" to such tempera-tures. The conclusion is clear: high temperature of itself is not a debilitating factor and the extremely low summer glycogen usually found in Barataria oysters is not an effect of temperature.

Several other studies designed to measure the capacity of Polydora to produce lethal effects on oysters were carried to completion. None of these did what they were designed for, even when precautions were taken to insure that incidence of infestation with Polydora was the only variable. All of these studies produced high mortalities in both controls and experimental oysters; sometimes it was higher in controls and sometimes lower. The reason for failure to obtain clear-cut results is obvious. Fungus disease as a major factor in production of oyster mortality so over-shadows lesser factors that the maximum effect of the latter is less than the normal

TABLE 2

SUMMARY OF DATA FROM A STUDY OF THE EFFECT OF POLYDORA
IN TERMS OF MORTALITY TO OYSTERS

	Control Oysters	Experimental Oysters	Date	Remarks
No. worms per oyster pre-exp.	77	360	July 1, 1948	
Original number oysters	802	833	July 1, 1948	
Dead, pre-exp.	36	63	July 9-10	Dewormed Exp. Oys.
Per cent	4.5	7.6	July 9-10	Placed on racks 7-10
Per cent per day	0.5	0.76		
Surviving	766	770	July 10	
Dead	14	61	July 27	
Per cent	1.8	7.9	July 27	
Per cent per day	0.1	0.46		
Surviving	752	709	July 27	
Dead	10	59	August 10	
Per cent	1.3	8.3	August 10	
Per cent per day	0.09	0.6	August 10	Dewormed second time
Surviving	742	650	August 10	on August 19
Dead	16	41	August 26	
Per cent	2.2	6.3	August 26	
Per cent per day	0.13	0.40	August 26	
Surviving	726	609	August 26	
Dead	36	75	September 23	
Per cent	5.0	12.3	September 23	
Per cent per day	0.18	0.44	September 23	
Surviving	690	534	September 23	Worm count: Exp.
Dead	41	36	October 19	542 per oyster;
Per cent	6.0	6.7	October 19	control 92 per
Per cent per day	0.23	0.26	October 19	oyster
Surviving	649	498	October 19	
Dead	14	24	November 11	
Per cent	2.16	4.8	November 11	
Per cent per day	0.094	0.21	November 11	
Surviving	635	474	November 11	
Dead	10	5	November 24	
Per cent	1.57	1.05	November 24	
Per cent per day	0.12	0.09	November 24	
Surviving	625	469	November 24	
Dead	15	13	December 16	
Per cent	2.4	2.8	December 16	
Per cent per day	0.11	0.13	December 16	
Surviving	610	456	December 16	
Dead	23	6	January 3, 1949	
Per cent	3.8	1.3	January 3, 1949	
Per cent per day	0.21	0.07	January 3, 1949	
Surviving	587	450	January 3, 1949	
Dead	6	9	January 11, 1949	Oysters stolen
Per cent	1.0	2.0	January 11, 1949	sometime be-
Per cent per day	0.13	0.25	January 11, 1949	tween Jan. 11
Total mortality	215	383		and Feb. 10 -
Per cent	27.0	46.0		3 trays only left

chance variation in the former. A relatively small mortality of, for example, 5 per cent caused by Polydora could easily be added to an 80 per cent mortality produced by Dermocystidium and just balance an 85 per cent mortality produced by the fungus alone in controls.

Experimentaion carried out outside of the epidemic disease area would not be conclusive. Such areas are low in Polydora infestation also. The same factors of environment which hold down the Polydora population would likely suppress also any lethal effects, and certainly it would not be possible to maintain high incidence in experimental oysters.

CONCLUSIONS

Studies on Polydora websteri show that:

1. Extremely heavy infestations occur in oysters in the Barataria Bay area of Louisiana.

2. Damage to shells and meats of oysters ordinarily occurs in summer months.

3. There is a cause and effect relation between Polydora and some of the yellow-colored ulcerations which are so common in the adductor muscle and on the visceral mass.

4. Under certain circumstances death of the oyster results from exceptionally rapid and widespread ulceration.

5. The extent of the mortality produced by Polydora cannot be accurately measured when overshadowed by a devastating disease such as that caused by Dermocystidium. Mortality produced by Polydora must necessarily be small but certainly exists.

6. Polydora sometimes attacks oysters in such numbers that destruction of a considerable part of the shell margin takes place. The significance of this in terms of mortality is not known.

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SPECIES OF NEMATOPSIS IN OSTREA VIRGINICA

By
Victor Sprague
Texas A and M Research Foundation

Nematopsis ostrearum Prytherch, 1940, contains two species which commonly occur simultaneously as spore stages in American oysters but which differ significantly in size of resistant spore, distribution in the oyster, size of gymnospore, and crab host specificity. Small spores, formerly considered immature stages, are approximately 10 by 14 microns and are typically most concentrated in the mantle tissues. Their corresponding vegetative stages, as Prytherch demonstrated, occur in the mud crabs Panopeus herbstii and Eurypanopeus depressus. Present studies show that Eurytius limosum, under experimental conditions, harbors the same parasite. The diameter of the gymnospores, an important diagnostic feature, is approximately 3 or 4 microns. For this species, previously described in detail, the name N. ostrearum is reserved. Large spores, formerly considered the mature stages of N. ostrearum but now known to represent a distinct species, are approximately 10 by 19 microns and have special affinity for the oyster gills. Their corresponding vegetative stages occur in the stone crab Menippe mercenaria. The gymospores are approximately 8 to 12 microns in diameter. Resistant spores and gymospores, both being larger than those of any other known species, and the decapod host specificity distinguish this from any other Nematopsis. Since only the resistant spores were previously described, the species they represent is here removed from N. ostrearum, in which it was inadvertently included, and named Nematopsis prytherchi n. sp. A third type of Nematopsis spore, approximately 7 by 11 microns, in oyster gills may be a form of the latter species.

STUDIES ON NEMATOPSIS. III.
N. OSTREARUM AND N. PRYTHERCHI WITH SPECIAL REFERENCE TO HOST-PARASITE RELATION

Victor Sprague and P. E. Orr

Nematopsis ostrearum Prytherch, 1940, has already been separated into two species by Sprague (1949) in an abstract anticipating publication of a series of papers dealing with Nematopsis. A second paper by Sprague et al. (manuscript) reports some observations and experiments made preliminary to the separation of the species. Herein are reported experiments concerning primarily host-parasite relation but also providing the final basis for separating the two species of Nematopsis. A later paper will deal more fully with morphology and life cycle of the parasites.

During June, 1948, through January, 1949, three sets of infection experiments were performed at Louisiana State University Marine Laboratory, Grand Isle, La., to obtain, in addition to other data, information on host-parasite relation. The first set of experiments was performed with two main purposes, (1) to test various decapods as possible primary hosts of Nematopsis species represented by various types of spores in oysters and (2) to determine whether oysters could be killed experimentally by producing in them heavy infestations of Nematopsis. Accordingly, this set is reported here in two parts. The first part is dealt with immediately below and the other is treated as the first of three mortality experiments described later.

DECAPODS TESTED AS POSSIBLE VECTORS OF NEMATOPSIS OF OYSTERS

Crabs and shrimp were collected from various localities in the vicinity of Grand Isle. All the Eurytium limosum and most of the Panopeus herbstii were collected from burrows among the mangrove roots on the bank of Bayou Rigaud near the Humble Company docks on Grand Isle. There large specimens, which proved more suitable for infection experiments than small ones, were found in abundance in the summer of 1948. Probably most of the Panopeus were P. herbstii forma obesa. Most of Eurypanopeus depressus, a small species, were taken from natural oyster reefs in Mudworm Bayou, Grand Terre Island. Petrolisthes armatus, another small species, was found in abundance on shell reefs at an oyster camp near Sugar House Bend in Barataria Bay. Menippe mercenaria, the largest of the crabs employed, was found in burrows among oyster shells, under stones, and in crevices among the debris found in certain localities in the intertidal zone. Most of those employed in the studies were collected on the mainland bank of Bayou Riguad not far from the bridge approaching the island. Many were also found around the ancient wreckage of a boat on the Gulf side of Grand Isle near Brown Hotel. This species was found in all sizes at the localities mentioned. Shrimp were collected anywhere in Barataria Bay or Bayou Rigaud by Trawling.

The different species of decapods, collected mostly in the early part of June, were segregated and the individuals of each species (exception Eurytium limosum, which was collected in July and included in the experiments in a special way to be described later) were separated into two equal lots. The different

lots of crabs and shrimp (about 25-100 in each lot, depending on numbers available and sizes of individuals) were placed in separate cypris aquaria. Chelae were removed from the larger crabs to prevent their causing damage to the cysters employed in the experiments. Removal was usually accomplished by crushing the chelae with pliers and thereby supplying a stimulus for autotomy. This method proved very satisfactory, whereas merely breaking off the chelae was often accompanied by excessive hemorrhage and death. In each aquarium with the decapods were placed 25-30 (depending on size) 1-2 year old oysters collected from Landry's beds at Chene Fleure, a low salinity area in Barataria Bay where the oysters were found to harbor relatively few Nematopsis spores (see Table II.) Oysters from the same source were placed in similar aquaria without decapods to serve as controls. It should be noted that in setting up the experiments decapods were employed without regard to whether they harbored gregarines at the time the experiments started (although some of them probably did). Although, ideally, the decapods should have been originally free of gregarines, this condition would have required more time and was not regarded as necessary for carrying out the purposes of the experiments which had to be completed in a limited time. Furthermore, separate experiments (described elsewhere) were performed when more rigid conditions seemed necessary to attain other ends (such as determining some of the time factors in the life cycle).

Running water was passed into the aquaria through aerating nozzels of a type devised by Mr. L. Boswell. The parts of each nozzle were a 4 inch length of 8 mm rubber tubing with four holes punched in radial arrangement near the middle, the glass portion of a medicine dropper and a glass T-tube approximately 8 mm in diameter. The side arm of the latter element was inserted into one end of a piece of rubber tube and the narrow portion of the medicine dropper was inserted into the other end. Then the broad portion of the medicine dropper was inserted into the water line. When properly adjusted these devices caused excellent mixing of air and water flowing through them into the aquaria. The rate of water flow through each of these aerators and into each aquarium was approximately 1900-2000 ml/min. Although the oxygen content of the water in the aquaria was not tested, it was assumed that the continuous rapid flow of aerated water kept the oxygen level sufficiently high to supply the needs of the experimental animals.

Each day (with few exceptions) the crabs and shrimp were fed large quantities (all they would consume) of whole oyster meats which, as occasional sampling indicated, contained large numbers of Nematopsis spores of the three types. The numbers of oysters used in the feeding is indicated by the fact that roughly a half bushel (before shucking) were fed almost daily throughout the summer of 1948. The oysters fed to the decapods were collected from two primary sources, Lirette's beds in Bay Ste. Elaine and natural reefs in Sugar House Bayou, Grand Terre Island.

The experiments, which (with the exception of that part involving Eyrytium) were started during the first week in June, were continued for about two months (except as otherwise indicated). During that time many of the decapods and oysters, experimental and control, were examined and the gregarines or N -topsis spores were observed. The results obtained with each decapodan apecies are given immediately below.

Penaeus aztecus Ives

When the shrimp were first collected examination of some of them showed (without exception, as in many collections examined during the previous year) the presence of numerous gregarines resembling Nematopsis. After feeding upon

infested oysters in the laboratory for a week or more they never harbored gregarines, as Table I indicates.

TABLE I

RESULTS OF EXAMINATION OF SHRIMP FOR THE PRESENCE OF GREGARINES
AFTER FEEDING UPON INFECTED OYSTER MEATS (7 June- 6 July)

Time in days between date of first feeding (7 June) and date of examination.	No. of shrimp examined	Gregarines present
7	2	0
8	1	0
9	5	0
14	1	0
18	2	0
22	2	0
25	5	0
28	5	0
29	10	0

Although the aporozoites readily emerged in the mid gut of the shrimp from Nematopsis spores ingested with the oyster meats, no later stages of the parasite were ever observed in these decapods. Furthermore, the shrimp lost the gregarines occurring naturally in them and apparently transmitted no parasites to the experimental oysters. These completely negative results point clearly to the conclusion that the gregarines occurring naturally in shrimp and the Nematopsis spores in oysters are different species. The gregarine of shrimp, observed many years ago by Prytherch (personal communication) was recently described by Sprague (in press) as a new species of Nematopsis.

Some of the oysters exposed to the shrimp were examined on June 11 near the beginning of the experiment (four days after the shrimp were first fed) and others on June 24 and 25. Nematopsis cysts in mantle, muscle and heart tissues were counted by treating the tissues with 10 per cent NaOH solution and employing a microscope fitted with a Whipple micrometer. In each such examination the cysts in 10 fields, each being 1 mm square and selected at random, were counted and the number of cysts per square mm was calculated. No method of counting the Nematopsis spores in the gills, where spores are unevenly distributed in dense clusters, was devised. The results of those examinations are given in Table II. Many control oysters (not exposed to the decapods) were likewise examined but it does not seem necessary to tabulate the results because they were like those indicated here. Table II, therefore, gives an accurate indication of the intensity of Nematopsis infection both in the control oysters and in similar ones at the beginning of the experiment and is employed as the basis of comparison with the intensities of infection in oysters after exposure to each of the species of decapods.

TABLE II

INCIDENCE OF NEMATOPSIS IN OYSTERS EXPOSED TO SHRIMP IN THE LABORATORY

Date Examined	No. of cysts/mm^2 in organs indicated			
	Margin of mantle	Heart	Muscle	Gill
June 11 (6 oysters)	3.0	0.0	0.7	Spores present
	0.3	0.0	0.1	None
	3.6	0.0	0.0	"
	0.3	0.0	0.0	"
	0.2	0.0	1.3	"
	0.5	0.0	0.0	"
June 24 and 25 (9 oysters)	0.3	0.0	0.1	Spores present
	2.4	0.4	0.4	None
	1.9	0.0	0.0	Spores present
	30.0	0.2	1.4	None
	0.8	0.0	0.0	Spores present
	0.7	0.0	0.0	None
	4.2	0.1	0.5	"
	0.3	0.0	0.1	"
	0.4	0.0	0.6	"

Petrolisthes armatus (Gibbes)

Two hundred crabs of this species were collected on June 4, 1948, at a shell reef in Barataria Bay north of Sugar House Bend. Six of them were examined on that day and the next and no gregarines were found. On June 7 and almost daily thereafter the crabs were fed infected oyster meats. Six of the crabs were then examined on July 6 and eight on July 28. All were found to be without Gregarines. Oysters exposed to the crabs about 2 months showed no increase of Nematopsis spores, the numbers remaining similar to those shown in Table II. It must be concluded that the results of the experiment provided no reason to suppose that P. armatus is one of the decapod hosts of species of Nematopsis observed in oysters.

Panopeus herbstii Milne Edwards

At the beginning of the experiment 69 small crabs were placed with 30 oysters in each of two aquarias. On June 18, 10 large crabs were added to each lot. In this part of the experiment only 4 of the crabs were examined after they had fed upon Nematopsis-infected oysters. Three of the four contained gregarines. The crabs were examined over a period of one to three weeks after the first day of feeding (June 9), a sufficiently long time (as demonstrated in other experiments) that gregarines which were found undoubledly developed from Nematopsis spores ingested during the experiment.

The Nematopsis counts in the exposed oysters at the beginning of the experiment (June 9) were very low, being presumably the same as those given in

Table II. After three weeks or more of exposure to the crabs 26 of 27 oysters examined contained enormous numbers of developing and mature spores in the mantles (Table III). The spores were mostly situated in a narrow band near to and parallel with the margin of the mantle. In one instance, not an unusual case, it was estimated that the concentration of cysts in that region was approximately 1000 (or 3500 spores) per square millimeter. Never were natural infections seen with more than about one third that intensity. Since the spores were usually present in such great numbers and since it was often difficult even to see the very slightly refractive immature ones, it was not feasible to try to count them. Counts were unnecessary for comparing with the numbers of cysts in control oysters, however, since the enormously greater numbers of spores (especially immature ones) in the experimental oysters was obvious and very striking. Even a few immature spores appeared in the gills but those (like the ones in the mantle, muscle and heart) were all of one type, the type which is most common in the mantle.

TABLE III

INCREASE IN NUMBERS OF NEMATOPSIS SPORES IN OYSTERS EXPOSED TO PANOPEUS HERBSTII,
(Exposure began June 9 and ended late in August)

Date of examination	No. of oysters examined	No. of oysters with obviously great increase of spores in the mantle	No. of oysters with obviously great increase of spores in the gills	No. of oysters with little or no increase of spores
June 11-28	9	0	0	9
June 29-July 15	7	6	0	1
July 22-Aug. 26	16	16	0	0
Sept. 28	4	4	0	0

The spores attributable to experimental infection were, when apparently mature, always of one type, being broadly rounded at the ends and measuring approximately 10 x 14-15 microns. They were probably identical (in spite of slight descrepancies in measurements) with spores which Prytherch (1940, p. 58) described as being 11-12 x 16 microns and which he designated as immature spores of Nematopsis ostrearum. Prytherch, who first produced those spores experimentally by exposing oysters to Panopeus herbstii and Eurypanopeus depressus, saw also larger spores (11 x 20 microns) which he considered as the final or mature stages of the same species. Since, in the present studies, only the smaller of those two types of spores developed in oysters exposed to Panopeus (the larger, as indicated by data presented later, developed only in oysters exposed to Menippe), it is believed that the larger spores which Prytherch observed during the course of this experiments were already present in his experimental oysters. It is also believed, therefore, that he inadvertently included two species under the name of Nematopsis ostrearum. Accordingly, the larger spores which Prythe. accidentally included in N. ostrearum have been removed to a new species and this name has been reserved for that species which he described in detail.

Eurypanopeus depressus (Smith)

Two hundred crabs of this species were collected on June 5 in Mudworm Bayou, Grand Terre Island. They were distributed evenly in two aquaria, each contain-

ing 30 Chene Fleure oysters and fed infested oysters from June 8 to the latter part of August. One of two crabs examined on the date of collection contained gregarines. Eight of 15 crabs examined between the dates of June 17 and August 21 contained gregarines, probably as a result of experimental infection. The incidence of Nematopsis in those crabs during the experiment was somewhat erratic, for reasons unknown, and the numbers were few. It seems quite possible that the size of the species was a factor, for Eurypanopeus is a small crab and is not capable of ingesting large quantities of food. It is reasonable to suppose, therefore, that the crabs often ingested few or no Nematopsis spores with the oyster tissues.

The oysters which were exposed to the crabs were presumed to have had at first essentially the same low intensity of Nematopsis infestation as those listed in Table II. When they were examined after several weeks of exposure to the crabs many showed an obviously greater number of spores in the mantle but not in the gills (Table IV).

TABLE IV

INCREASE OF NEMATOPSIS SPORES IN OYSTERS EXPOSED TO EURYPANOPEUS DEPRESSUS.
(Exposure began June 8, 1948 and ended late in August)

Date of examination	No. of oysters examined	No. of oysters with obviously great increase of spores in the mantle	No. of oysters with obvious increase of spores in the gills	No. of oysters with little or no increase of spores
June 11	4	0	0	4
June 25	4	1	0	3
July 6-Aug. 6	5	2	0	3
Aug. 9-Sept. 25	40	40	0	0

Some of the spores produced experimentally in the oyster mantle s appeared somewhat smaller than those usually seen in instances of natural infestation and tended to be pointed at one end. They closely resembled the Nematopsis spores which are common in Modiolus demissus occurring on Grand Isle. It is futile to speculate on the significance of those unusual spores without further study. The great majority of the spores, on the other hand, were indistinguishable from those transmitted by Panopeus. It is concluded, therefore, that the findings of Prytherch (1938, 1940) that Eurypanopeus depressus is one of the normal decapodan hosts of Nematopsis ostrearum was probably confirmed. It is further concluded that this crab species does not transmit the large spores, N. prytherchi, which are characteristically seen in oyster gills.

Eurytium limosum (Say)

On July 15, 1948, 215 large crabs were collected from among the mangrove roots along the bank of Bayou Rigaud near the Humble Company plant on Grand Isle. Twenty-three of them (10.7 per cent) proved to be Eurytium limosum and the rest Panopeus herbstii. Since the former species was found in such relative abundance and since, according to Rathbun (1930), it is "related to Panopeus and its allies",

it was tested by means of an infection experiment to determine whether it might harbor Nematopsis and transmit this parasite to oysters.

Unfortunately, none of the crabs was examined before they were fed infested oyster meats. It is not known, therefore, whether they harbored gregarines naturally. The crabs were all placed in an aquarium with 12 oysters, presumed to have Nematopsis counts similar to those in Table II, and similar oysters in another aquarium without crabs were employed as controls. The crabs were fed Nematopsis-infested oysters daily (starting July 17) and examination of crabs and oysters was made according to the schedule given in Table V. As the data show, all the crabs examined contained many gregarines (either trophic stages or gamontocysts or both). The trophic stages in the midgut and the gamontocysts (a term used by Filipponi, 1949, for the older "gametocysts") attached to the lining of the rectum near the anus could not be distinguished from those occurring in Panopeus herbstii. The gymnospores (which are very significant in characterizing species and which will be described later) of the gregarines in Eurytium appeared identical with those in Panopeus. Furthermore, the Nematopsis spores which developed in the mantles of the oysters exposed to the crabs appeared to be those of Nematopsis ostrearum.

TABLE V

EXPERIMENTAL INFECTION OF EURYTIUM LIMOSUM AND OYSTERS WITH NEMATOPSIS OSTREARUM

No. of days after start of feeding (July 17)	Crabs			Oysters		
	No. examined	Gregarines present		No. examined	Spores in	
		Trophic	Cysts		Mantle	Gill
1	1	Many	0	-	-	-
2	1	Many	9	-	-	-
3	2	Many,Few	4,ca.80	-	-	-
5	-	-	-	1	no increase	no increase
6	2	Few,None	6,ca.200	-	-	-
11	1	Many	ca.50	1	no increase	no increase
13	-	-	-	1	no increase	no increase
18	-	-	-	1	many young	no increase
20	-	-	-	6	many young	no increase
20	-	-	-	1	no increase	no increase
20	-	-	-	6	(controls) no increase	
23	-	-	-	1	many young and mature	no increase

Although it is not known whether the crabs employed in the experiment harbored gregarines in the beginning, it is believed (because of time and temperature factors involved and because gregarines were observed in all stages of development) that most of those observed were acquired in the laboratory. That belief was supported by another phase of the experiment carried out as follows: When it seemed evident that Nematopsis was developing in Eurytium in the laboratory the remaining crabs were starved for a week and two of them then examined were found negative. The others were fed daily upon infested oysters and further examinations were made. After feeding the crabs were found to harbor gregarines as shown in Table VI. Again the morphological features of the parasite (sporadins,

gamontocysts, gymnospores, resistant spores) appeared identical with those
of Nematopsis ostrearum. There seems to be very little doubt, therefore, that
infection of Eurytium limosum was experimentally produced in the laboratory
and that the infection was further transmitted to oysters. Since the entire
life cycle of the parasite was completed under laboratory conditions, another
decapod host of Nematopsis ostrearum was added to the list of those already made
known by Prytherch.

TABLE VI

OCCURRENCE OF NEMATOPSIS IN EURYTIUM LIMOSUM STARVED FOR A WEEK AND THEN FED
INFESTED OYSTERS

Date	No. of crabs examined	Gregarines present	
		In midgut	In rectum
Aug. 18 (after week starving)	2	0	0
Aug. 19 (first feeding)	-	-	-
Aug. 20	1	many large	0
Aug. 22	1	many large	4 young 'cysts
Aug. 28	1	many	ca. 50 'cysts, all stages

Menippe mercenaria (Say)

These crabs were fed Nematopsis-infected oyster meats on June 9 and almost
daily thereafter during the course of the experiment. Ten of them were ex-
amined (between one week and two months after the start of feeding) and all
contained gregarines which were probably acquired in the laboratory. All the
major developmental stages, sporozoites to mature gamontocysts with gymnospores,
were seen in great numbers. The majority of the stages observed were not noti-
ceably different from corresponding stages of Nematopsis ostrearum seen in
Panopeus herbstii, Eurypanopeus depressus and Eurytium limosum. The gymnospores
seen in Menippe (in this and other feeding experiments) were, however, always
uniformly and very conspicuously larger than those seen in the other crabs.
This fact strongly supports others which point to the conclusion that Menippe
harbors a species of Nematopsis other than N. ostrearum (restricted sense).

Oysters from Chene Fleure, presumed to have Nematopsis counts similar to
those given in Table II, were exposed to the crabs for varying lengths of time
(beginning June 11) up to more than two months. During June (beginning June
18), July and August 36 of the oysters were examined microscopically. The gills
of those oysters, without exception, contained large numbers of Nematopsis spores,
mostly immature and usually of the two types commonly seen in naturall, infested
gills. The great majority were, however, of the larger type. In many instances
the entire gill appeared to be literally saturated with spores, each microscopic
field containing apparently many thousands. The intensity of the infestation was
incomparably greater than any seen in nature. Appreciable, but not very great,
numbers of similar spores were often observed in mantle, muscle and heart tissues;
but no increase in numbers of the type usually observed in these organs was noted.
The distribution of the spores developing in the mantle was also noteworthy.

Unlike the usual spores in mantle tissue, they were not concentrated in a band parallel with the margin but were scattered, sometimes in clusters, throughout the mantle tissue.

Since oysters fed to Menippe contained (in addition to others) typical spores of Nematopsis ostrearum but similar spores did not develop in oysters after exposure to this crab, it is presumed that N. ostrearum was not capable of developing in Menippe. The spores which were transmitted are, furthermore, presumed to represent a different species.

From the experiments and observations already recorded here four significant types of evidence have emerged which, taken together, strongly support the conclusion that the large Nematopsis spores having special affinity for oyster gills and the smaller ones having special affinity for mantle (which Prytherch designated as respectively mature and immature stages of N. ostrearum) represent two distinct species. Those types of evidence are: (1) morphological differences in resistant spores; (2) morphological differences in gymnospores; (3) differences in decapod host specificity; (4) marked differences in distribution of spores in the molluscan host. It has already been stated that the name Nematopsis ostrearum probably should be reserved for all the stages which Prytherch described exclusive of that which he designated as the mature spore. In a preliminary note Sprague (1949) has already given the latter stage a new name, N. prytherchi, in honor of Dr. Herbert F. Prytherch who first observed it.

Although the authors believe the evidence presented above justifies the separation of species, they recognize a serious flaw in the experiments which permits another possible interpretation of the results. Namely, it was necessary, because the only oysters available for feeding the crabs had mixed infestations, to feed more than one kind of spore at the same time. When morphologically different parasites developed in the different crabs and those gave rise to morphologically and physiologically different spores in oysters, it was assumed that there was a selective affinity between the crab species and the different infective stages of the parasites which they ingested. Therefore, the evidence presented above rests on an assumption (not completely unfounded, as Sprague et al. have indicated in a previous paper, in manuscript) rather than on established fact. A possible alternative assumption is that the different types of spores developed indifferently in the various crabs. This would suggest that the differences seen in the parasites could possibly be differences within a single species caused by passage through different crab hosts. The conclusions reached here, therefore, require confirmation by further experimentation employing more suitable materials.

RELATION OF THE PARASITES TO THE MOLLUSCAN HOSTS

Certain aspects of the host-parasite relation, particularly the determination of the decapodan hosts of Nematopsis ostrearum and N. prytherchi, have already been considered. The rest of this paper deals more with the interaction between host and parasite but expecially with the effect of the parasite on the molluscan host. The latter was studied by means of a series of infection experiments in which an attempt was made to introduce into the oysters lethal numbers of Nematopsis spores.

First mortality experiment

As already stated, the first mortality experiment was one phase of that experiment reported above and designed also to test various decapods as possible hosts

of the species of Nematopsis under consideration.

Oysters exposed to infected crabs in this experiment were from Landry's beds in Chene Fleure and contained relatively few Nematopsis spores, the numbers being as shown in Table II. They were of three lots which had been handled differently after collection and must be considered separately. Lot 1 oysters were collected June 9, 1948, and taken to the laboratory on that date. For two or three days thereafter there was high mortality among those oysters attributable, possibly, to rather sudden and drastic changes in environment. After those early mortalities they seemed to be a healthy lot of oysters. Lot 2 oysters were collected on March 5, 1948, by McLendon and taken to the laboratory where they were stored for future use. About the middle of April they were transferred to Bayou Rigaud for storage because of shortage of space in the laboratory. On June 9 they were returned to the laboratory for use in the experiment reported here. Oysters of lot 3 were the same as lot 2 except that they had not been taken to the bayou; they had been stored in the laboratory since they were collected on March 5.

The experiment was set up between the inclusive dates June 6 and June 11, 1948. Twenty-five or 30 oysters (depending on size) were placed in each of several (18" by 24" by 10") wooden aquaria with running sea water. The water was aerated in this and the following experiments as already described. Two of the awuaria were employed for oysters exposed to each of the crab species and one each for the different lots of oysters (without crabs) was used for a control. Chelae were removed, when they were large, from the crabs which were placed in the aquaria and these animals were fed daily large quantities of Nematopsis-infested oysters. The experimental oysters were examined daily and deaths noted.

It was assumed that most of the oyster mortalities during the first few days may have been attributable to the changed environment. Oysters which died before June 18 were, therefore, simply replaced and records of mortalities were kept beginning with that date. A few living oysters from each aquarium were examined during the course of the experiment and these were deducted from the original numbers before mortality percentages were calculated. Most of those which died were examined microscopically. Those exposed to the crabs, as already indicated, contained unusually large numbers of Nematopsis spores but the controls did not. The mortality records from June 18 to August 30, 1948, are shown in Table VII.

TABLE VII

RESULTS OF MORTALITY EXPERIMENT NUMBER 1

Crabs employed	Species of Nematopsis introduced into the oysters	Oysters			
		Lot No.	No. employed	Mortality	
				No.	Percent
None	None (control)	1	21	4	1.0
Menippe	N. prytherchi	1	44	40	90.9
None	None (control)	2	27	19	70.4
Panopeus	N. ostrearum	2	56	33	58.9
None	None (control)	3	28	10	35.7
Eurypanopeus	N. ostrearum	3	54	16	29.6

Second mortality experiment

Since the first experiment gave data of questionable significance, another was performed with the hope that some of the complicating factors might not enter into it. T... experiment was likewise complicated by unknown mortality factors and the results are of doubtful value. It is, therefore, presented very briefly here.

Oysters employed were the largest obtainable and were collected from two sources with respectively low and high incidence of Nematopsis. The object was to test the possible effect of heavy experimental infestation of Nematopsis on large oysters which had not been previously heavily infested and on others which already harbored relatively large numbers of spores naturally. The first source was Bassa Bassa, in Barataria Bay, where the natural incidence of Nematopsis was relatively slight. The oysters were collected by J. G. Mackin (date unknown to the authors) and stored in trays in the same locality till they were taken to the laboratory on July 3, 1948, for use in the experiment. The other oysters were originally from Cheramie's beds in Barataria Bay and harbored numerous Nematopsis spores. They were collected (date unknown to the writers) by J. G. Mackin and stored in Bayou Rigaud till July 2, 1948, when they were taken to the laboratory.

The oysters were distributed in large wooden aquaria with aerated running sea water. Some were exposed to Nematopsis infested crabs and others, without crabs, were considered as controls. The experiment was begun on July 6 and terminated on September 20, 1948. As shown in Table VIII, most of the control oysters died. Apparently, mortality factors other than Nematopsis were operative. The nematopsis, parasites possibly contributed to the deaths of the oysters exposed to the crabs, however, because they all died and most of them showed a great increase in number of Nematopsis spores.

TABLE VIII

RESULTS OF MORTALITY EXPERIMENT NO. 2

Crabs employed	Species of Nematopsis introduced into oysters	Oysters			
		Source	No. employed	Mortality	
				No.	Percent
None	None (control)	Bassa Bassa	64	57	89.1
Menippe	N. prytherchi	Bassa Bassa	49	49	100.0
Panopeus	N. ostrearum	Bassa Bassa	50	50	100.0
None	None (control)	Cheramie	80	73	91.2
Menippe	N. prytherchi	Cheramie	80	80	100.0
Panopeus	N. ostrearum	Cheramie	80	80	100.0

Third mortality experiment

Twenty-five Chene Fleure oysters, as nearly uniform in size as possible and acclimated to laboratory conditions for one week, were placed in each of 2 aquaria (18" x 24" by 10") with running sea water aerated as already described. With them in each tank were placed 25 Menippe mercenaria with chelae removed. The crabs were fairly uniform in size, varying within a range of 12mm. Almost

daily the crabs were fed Nematopsis-infested oysters obtained from Lirette's
beds in Bay Ste. Elaine and Staufflet's beds in Bayou Bas Bleu. Two other
aquaria, for controls, were set up in identical manner except that the crabs
were previous y starved for a week to rid them of Nematopsis and were thereafter
fed only small fish and shrimp. Every attempt was made to maintain identical
conditions in the experimental and control aquaria excepting the one factor,
Nematopsis prytherchi. Two similar experimental and two control tanks were set
up with Panopeus herbstii to test the possible effect of Nematopsis ostrearum on
the oysters.

Each day (with few exceptions an inventory was made of the crabs and oys-
ters, to note mortalities, and the water temperature and rate of flow was re-
corded. Most of the oysters were examined microscopically after they died and
the incidence of Nematopsis noted. The experiment was carried out over a period
of two and one half months (Nove. 1, 1948, to Jan. 15, 1949). The mortality data
are shown in Table IX.

TABLE IX

RESULTS OF MORTALITY EXPERIMENT NO. 3

Kind	Crabs			Species of Nematopsis introduced	Oysters		
	No. employed*	Mortality			No. employed	Mortality	
		No.	Percent			No.	Percent
Menippe	54	4	7.4	None (control)	50	11	22.0
Menippe	83	33	38.4	N. prytherchi	50	19	38.0
Panopeus	59	9	15.3	None (control)	50	9	18.0
Panopeus	61	11	18.0	N. ostrearum	50	24	48.0

* The number of crabs in each tank was kept constant (25) by replacing those which
  died. The numbers given in this table are combined totals for two tanks.

It will be noted that, for both species of the parasite, there was some-
what higher mortality among experimentally infested oysters than among the con-
trols. Those differences, although not large, support the slight evidence ob-
tained in the other experiments which points to the conclusion that the heavy ex-
perimental infestations of Nematopsis probably contributed to the oyster mortali-
ties. It is noteworthy, also, that there was slightly higher mortality among
the infested crabs than among the controls. It is possible the parasites have
some deleterious effect on the decapodan hosts.

Discussion of mortality experiments

It is unfortunate that Nematopsis-free oysters were not available for ex-
perimental purposes so that mortality among them could be compared with that of
experimentally infested ones. Such a comparison might have yielded results signi-
ficantly different from those obtained in the experiments reported here. In
practice it was necessary to superimpose an experimental infestation upon a natural
one and to hope that the results would be susceptible of interpretation. Although

experimental infestations were usually incomparably more intense than natural ones, their effects cannot be decided with confidence because, in addition to other reasons, the effects of the spores already present is unknown. One must admit that among various possible effects of relatively few spores initially present are the following: (1) They might have no appreciable effect. This assumption was necessarily the basis of some of the experimental work, since it was presumed that much heavier infestations produced experimentally might be expected to produce significant results. (2) They could be essentially as lethal in effect as the heavier infestations, in which case the spores superimposed upon them would not cause a greatly increased mortality rate. (3) They could have an immunizing effect, in which case their hosts would be immune survivors of previous epidemics and possibly very resistant to superimposed infestations. In any case, the initial presence of Nematopsis spores, however few they may have been, complicated the experimental results so that conclusions as to the effects of the experimental infestations cannot be made with confidence.

It seems highly probable that the experiments were complicated also by highly lethal factors other than Nematopsis. Such factors could seriously obscure any mortality effect of the latter. It cannot be confidently concluded, therefore, in view of this and considerations in the above paragraph, that the mortality experiments have either confirmed or refuted Prytherch's (1940) view that Nematopsis is highly lethal to its molluscan host. Yet in each of the three experiments there were some data suggesting that Nematopsis possibly contributed to mortality. On the other hand, the fact of the survival of many oysters in spite of very heavy infestations may suggest that this parasite is not highly lethal. One must, therefore, admit that extremely heavy experimental infestations of Nematopsis may be deleterious to oysters but, at the same time, one may seriously doubt that the numbers of spores naturally occurring in oysters contribute very significantly to mortality.

Distribution of spores in the oyster

Spores of Nematopsis prytherchi may occur in blood cells in almost any of the organs of the molluscan host. They have been seen in appreciable numbers in labial palps, mantle, heart, adductor muscle, and among the liver tubules. They are by far most abundant, however, in the blood vessels of the gills, particularly those in the proximal region of those organs. In sections the hypertrophied phagocytes containing spores seem to adhere to the walls of the blood vessels and to each other. This adhesion may explain the observation of Prytherch (1940, p. 51) that "many of the gymnospores do not migrate far from the point of entrance." As these cysts (blood cells containing spores) accumulate they form an increasingly thick layer on the walls of the vessels and the passageway for the circulation of blood becomes correspondingly smaller. In cases of very heavy infestation some of the vessels may become completely obliterated. This narrowing of the blood vessels, or their complete occlusion, would seem to be a serious impediment to the blood circulation and, therefore, to respiration.

There has been some question, during the early phases of the investigation, whether the mature spores of N. prytherchi are intracellular. In bulk samples of fresh gill tissues they appear as independent spores packed together in large masses, unlike those of N. ostrearum which are contained within highly granular and very conspicuous host cells ("cysts") somewhat scattered in the involved tissues. Sections show plainly, however, that the mature spores are contained within cell membranes which seem to be the principle remnants of greatly hypertrophied and otherwise modified phagocytes. Those cell membranes, being the

remnants of agglutinated phagocytes, remain attached to one another and, in sections, appear as a continuous reticulum containing the spores in its interstices. This may explain why the spores which would, if free, be carried by the blood circulation to all parts of the body, remain stationary.

N. ostrearum and only one other previously described species (Nematopsis sp. Schneider, 1892) have special affinity for the mantle of the molluscan host, all others being most highly concentrated in the gills. This statement does not take into account spores reported by Sprague (in press) in mantle and gill of Modiolus demissus and others in mantle of Ensis minor because that report was based on very casual observations. The cysts of Nematopsis ostrearum are concentrated in a narrow band, roughly 2 mm wide, near to an parallel with the margin of the mantle. They do not occur in clumps, as do those of N. prytherchi, but are rather uniformly distributed within the band. Limited observations on sectioned material failed to reveal the reason for the peculiar concentration of cysts. Cysts in the mantle are not limited to that part already mentioned but may occur in any region, even in the tentacles, in much smaller numbers. They also occur in considerable numbers in adductor muscle, heart, gill, labial palps, and perhaps any other organ. The results of counts of cysts in mantle, muscle and heart of several oysters suggested that there is little or no correlation between the numbers of cysts in the different organs.

From the foregoing discussion it is apparent that both species of Nematopsis under consideration are widely distributed in many organs of the molluscan host. It may, therefore, be an over statement to say without qualification that one has special affinity for mantle and one for gills. The statement may not be strictly accurate excepting when only mantle and gill are compared with respect to the spores which infest them.

Host Specificity

Previous investigators have held the view that a given species of Nematopsis is capable of invading the tissues of a considerable number of different mulluscan species. Thus, Leger and Duboscq (1925), Hatt (1927, 1931) and Prytherch (1938, 1940) each listed many molluscs supposed to be hosts respectively of N. portunidarum, N. legeri and N. ostrearum. Those lists seem to have been compiled largely on the basis of casual observation of somewhat similar spores occurring naturally in the various mulluscs. One investigator, Hatt (1931), believed he had demonstrated experimentally that N. legeri, a normal parasite of Mytilus galloprovincialis, could infest a mollusc other than its usual host. The present writers arrive at just the opposite conclusion from Hatt's experiment, since Hatt was able to obtain only very slight development of the parasite in several weeks in the unnatural host, M. minimus. It is suggested here that more careful comparison of the Nematopsis spores occurring in different molluscs, combined with critical infection experiments, might result in the recognition of several other distinct species of Nematopsis and lead to a modification of the generally held opinion that each of these parasites is capable of developing to the mature spore stage in a great variety of mulluscs.

RELATION OF THE PARASITES TO THE DECAPODAN HOSTS

Excepting the studies to determine which hosts harbored each species of Nematopsis investigated, the relation of parasite and decapodan host was noted only casually. Such limited observations as were made are mentioned at this time.

Effect of parasite on host.

During the course of the experiments described above numerous crabs were subjected to repeated heavy infestations over a long period of time. There were occasional mortalities attributable to mechanical injury or, when the water flow stopped for a few hours, to asphyxiation. In only one experiment was a record of the crab mortalities kept. In that instance, as already pointed out, there was somewhat greater mortality among the experimental animals than among the controls (Table IX). Although the data are very limited, the gregarine parasites possibly contributed to those mortalities.

Distribution of the parasites in the crab.

Vegetative stages of Porosporidae, as well as other gut-inhabiting gregarines, are characteristically situated in the anterior portion of the mid-gut. Young trophozoites in this family are attached to epithelial cells by means of spherical epimerites. Later trophic stages are free in the lumen. Gamontocysts are cemented to the chitinous lining of the rectum. The location in the rectum is a characteristic of the species, some species (Porospora gigantea, P. nephropis, Nematopsis prytherchi and N. penaei) being distributed along the entire length of this organ and others (N. ostrearum, N. legeri, N. naraisi and N. portunidarum) being concentrated at the extreme posterior end near the anus. The particular distribution of the parasites in the rectum probably depends on particular environmental condition corresponding to the specific requirements of the parasite. It may be that those species situated at the anus require for their development some contact with sea water. More complete dependence upon the sea water environment for gamontocyst development is illustrated by Carcinoectes conformis, a crab inhabiting gregarine possibly related to the Porosporidae and studied by Ball (1938). In this species the gamontocysts are usually found attached to the telson and various abdominal appendages of the host.

Host specificity

The findings with respect to host specificity of the parasites under consideration have already been presented and need not be repeated at this time. Suffice it to say that they are in agreement with those of previous investigators who have found that the spores will germinate indifferently in many species of decapods but the sporozoites can survive and continue their development only in one or few host species. For most of the Porosporidae, including Nematopsis prytherchi, only one decapodan host is known for each species. N. ostrearum, however, has at least three decapodan hosts (all of different genera) and N. portunidarum, according to Leger and Duboscq (1913), has two or possibly three. It is quite probable than N. penaei, as pointed out by Sprague (in press), inhabits more than one species of Penaeus.

A GENERAL CONSIDERATION OF GREGARINES AS PARASITES

Parasitism, in a strict sense, refers to that relationship between two individuals in which one, the parasite, is benefited by living on or in the other, the host, to the detriment of the latter. The effect of the parasite on the host is the resultant of many interacting factors and ranges from negligible to lethal. The cephaline gregarines generally are not noted for being highly detrimental to their hosts, the best known effect being damage done to the epithelial cells to which the young trophozoites are attached in the intestine of the host. This effect, whatever it may be, is directly related to the number

of parasites present, since each parasite attacks only one host cell. The
number present at a given time is limited by two important factors inherent in
the process of reproduction. In the first place, the Eugregarinina (to which
category Nematopsis belongs) are peculiar in having no asexual reproduction.
The vast majority of the parasitic Protozoa (as well as pathogenic bacteria)
undergo some type (or types) of asexual reproduction (binary fission, multiple
fission, budding, plasmotomy, etc.) to produce large numbers of new individuals
like the parents which are capable of spreading the infection within the body
of the host. These unique Protozoa, the Eugregarinina, reporduce only by
progony (sexual reproduction) and the products leave the body of the host to
perish or enter again the same or another host. Thus, these parasites do not
increase numerically within the host by reproduction. In this respect they are
comparable with many helminth parasites rather than with other Sporozoa.
Parasitism of this type, as Chandler (1949) has pointed out, is frequently termed
"infestation" to distinguish it from "infection", that type of parasitism in
which the invaders increase in numbers within the host by multiplicative repro-
duction. Unlike the helminths, however, the adult gregarines themselves perish
in the process of sexual reproduction, this being the second limiting factor,
mentioned above, in the numbers of parasites in the host. Therefore, the repro-
ductive process in these gregarines not only fails to increase but actually de-
creases the numbers of individuals present in the host. This process causes
complete and rapid elimination of the gregarines present at a given time and if
the infestation is maintained it must be replenished by new invaders. The num-
ber of parasites (and possibly their effect), therefore, fluctuates and the
severity of their effect may depend very largely on whether or not there is re-
peated and frequent acquisition of new parasites. Acquisition of great numbers
possibly results in damage to the host other than the destruction of epithelial
cells as mentioned above. It is probable that the sheer bulk of numbers may
damage the intestine mechanically or interfere with its proper function. There
may be also a toxic effect of the parasites, a debatable subject discussed by
Watson (1916).

The above remarks apply either to certain gregarines which have only one
host or to those stages of the Porosporidae occurring in the definitive host
(the one in which sexual reproduction occurs, the decapodan host of Porosporidae).
In Nematopsis the possible effect of the parasite on the intermediate (molluscan)
host is of particular interest. Here, again, there is no multiplication within
the host but each individual parasite (spore) infesting the oyster is acquired
as an original invader. Any effect of the parasite on the host must, therefore,
be caused by those original invaders; there can be no effect (as in most Sporozoa
and pathogenic bacteria) attributable to multiplication of the parasite within
the oyster. Spores of Nematopsis, unlike the vegetative stages, are as Landau
and Galtsoff (1951) have recently confirmed, cumulative. This suggests the
possibility of a cumulative effect on the host. Actually, as already suggested,
spores of N. prytherchi may have such an effect when they accumulate in great
numbers and cause progressive occlusion of the blood vessels of the gills and
possibly impede the exchange of gasses involved in respiration. Another possible
detrimental effect on the oyster might result if toxic end products of metabolism
were liberated into the host tissues by developing sporoblasts, although the au-
thors have not tested this hypothesis.

SUMMARY AND CONCLUSIONS

Experiments are reported here which provided a partial basis on which
Nematopsis ostrearum Prytherch 1940, has already been separated into N. ostrearum
Prytherch, emended, and N. prytherchi Sprague, 1949.

N. prytherchi includes only one stage described by Prytherch, large spores which are by far more common in oyster gill than mantle and which Prytherch mistakenly regarded as final developmental stages of spores belonging to the species he described in detail. Vegetative stages of N. prytherchi occur in the crab Menippe mercenaria.

Nematopsis ostrearum, emended, includes smaller spores, which are rare in oyster gills but common in mantle and other organs, and vegetative stages which Prytherch described in the crabs Panopeus herbstii and Eurypanopeus depressus. The experiments herein reported demonstrated that Eurytium limosum is also host to this gregarine.

Experiments failed to produce any evidence that either the crab Petrolisthes armatus or the shrimp Penaeus aztecus is host to the species of Nematopsis found in oysters, although the shrimp always harbors naturally a gregarine resembling Nematopsis.

Extremely heavy infestations of both species of Nematopsis were introduced separately into oysters in a series of experiments attempting thereby to kill the oysters. The results were complicated by unknown mortality factors and were not consistent or decisive. Still, there was a trend toward greater mortality among oysters into which Nematopsis was introduced than among controls. It must be admitted, therefore, that the experimental infestations, incomparably greater than any known in nature, may have cortributed to the mortality of the oysters. On the other hand, there is still room to doubt that the numbers of spores occurring naturally in oysters contribute significantly to mortality.

Very limited data suggest that Nematopsis may, in some instances, be significantly deleterious to the decapodan host.

Increase of numbers of Nematopsis parasites in the decapodan or, so far as is known, in the molluscan host is never by multiplicative reproduction within the host but by only new invasion.

From the decapodan host the parasites tend to be rapidly and completely eliminated, rather than increased, by the reproductive process. If there is replenishment it is by new invasion and the severity of any deleterious effect on the host may depend largely on whether there is frequent acquisition of large numbers of new parasites.

In the molluscan host the number of parasites is cumulative. This suggests the possibility that any deleterious effect on the host may be related to numbers of parasites acquired over a period of time.

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"OBSERVATIONS ON THE OCCURENCE OF THE OYSTER CRAB, PINNOTHERES OSTREUM, AS RELATED TO OYSTER DAMAGE IN DELAWARE BAY"

by

Franklin B. Flower
and
John J. McDermott

Oysters in Delaware Bay are frequently infected with the oyster crab, Pinnotheres ostreum.

The first or invasive stage of both the male and female are small hard shelled crabs having a carapace width of about two to three millimeters. The female crab goes through four more stages of development until it reaches the fifth and final stage having a carapace width from six to fifteen milli- meters. The male oyster crab goes through only one more stage of development to become a second stage crab with a soft carapace and but little larger than the first stage crab.

We are using the stage designations given the oyster crab by Stauber during his work with them in Delaware Bay in 1941 and 1942.

All stages of the male and female oyster crabs except the first have a soft carapace. The fifth stage female crab is the largest and it is this stage that produces the young.

Due to their small size, the males and first three stages of the female are commonly called "lice" by the oystermen, while they commonly refer to the fourth and fifth stage females as "oyster crabs."

Inside the oyster Pinnotheres often causes gill erosion of varying degrees. The damage caused by the crabs may result directly or indirectly in the death of large numbers of oysters as noted by Stauber in Delaware Bay in 1941.

Last fall the New Jersey oystermen of Delaware Bay found large numbers of dead market size oysters that had apparently been in good condition at midsummer. No apparent cause of death was noted except that large numbers of Pinnotheres were found in the oysters still on the beds. In fact, the numbers of fifth stage Pinnotheres found in some of the shucking houses were numerous enough to make it profitable to can oyster crabs as well as oysters.

Because of the pressure of other duties quantitative studies of Pinnotheres on the oyster beds were not begun until last winter. These ex- aminations have been continued to the present time (first of August, 1952) with the greatest intensity of examination being made this summer. Our examinations have been confined to the natural and leased bottom on the New Jersey side of Delaware Bay. For the purpose of comparison the oyster grounds were arbitrarily divided into three areas according to relative salinity. These areas are as follows:

Lower Salinity Area - Those oyster beds on the natural beds above the Cohansey River Inlet.

Medium Salinity Area - The rest of the upbay natural beds, from the Cohansey River Inlet to the Southwest line, plus the natural and leased beds

in Maurice River Cove above a line from $\frac{1}{2}$ miles NE of Egg Island Point to the mouth of Goshen Creek on the Cape May peninsula.

Higher Salinity Area - The rest of the oyster grounds on the New Jersey side of Delaware Bay are included in this area. Most of the leased grounds are in this area.

A summary of the results of the winter (December thru February), spring (April), and summer (June and July) examinations of the oyster beds for the presence of oyster crabs in the oysters and gill damage to the oysters are given in the table below.

Examination Season	Area Examined	Salinity Range		% of Oysters Showing Oyster Crab Gill Damage	% of Oysters Containing Oyster Crabs
		Maximum Greater-Than	Maximum Less-Than		
Winter	Lower Salinity	-	12-13ppt	40	20
"	Medium Salinity	12-13ppt	20ppt	50	20
"	Higher Salinity	20ppt	-	60	35-40
Spring	Lower Salinity	-	6ppt	40	10
"	Medium Salinity	6ppt	about 16ppt	50	20
"	Higher Salinity	about 16ppt	-	60	35-40
Summer	Lower Salinity	-	15ppt	40	2
"	Medium Salinity	15ppt	20ppt	50	20
"	Higher Salinity	20 ppt	-	60	35-40

The winter examinations showed 20% of the oysters in the lower salinity area to contain oyster crabs. In the spring only 10% of the oysters in this area contained Pinnotheres, while by summer only 2% of the oysters contained crabs. At the same time the percentage of oysters containing Pinnotheres in the medium and higher salinity areas remained the same at 20 and 35 to 40 percent respectively. The reduction in the percent oysters containing oyster crabs in the lower salinity area evidently occurred at the time of salinity reduction during the spring runoff.

The percent of oysters showing oyster crab gill damage in the lower, medium, and higher salinity areas has remained at 40, 50 and 60 percent throughout the three examination periods.

We believe that the reduction in the percent oysters containing Pinnotheres in the lower salinity area during the spring fresh water runoff indicates that Pinnotheres has less tolerance for low salinity than the oyster. We also believe that the reduction in the percent oysters showing Pinnotheres gill damage and containing live Pinnotheres which occurred with decreasing salinities indicates that Pinnotheres has less tolerance for low salinity than the oyster.

In only a very few cases did we find more than one crab in an oyster, and in no case were more than two crabs found in a single oyster.

The first ovigerous females were noted about the middle of June when water temperatures were about $75^{\circ}F$. During July about three-fourths of all fifth stage females found were ovigerous. The first fifth stage females containing zoea were noted about the middle of July when water temperatures were about 80 to $82^{\circ}F$. However, we believe that we could have missed seeing the first zoea. Sandoz and Hopkins report that in the laboratory it takes thirteen days for the egg to develop into the first zoea.

As of August 1 we had not noted any significant invasion of oysters by first stage crabs, but the indications are that we will be finding this sometime during August.

In summary we wish to say:

1. This series of preliminary observations indicates that Pinnotheres ostreum may have less tolerance for low salinity water than the oyster.

2. There has been a heavy infestation of Delaware Bay oysters by Pinnotheres during the past year.

3. Ovigerous fifth stage females have been observed since the middle of June, and during July three-fourths of all fifth stage females were ovigerous.

PRELIMINARY STUDIES OF THE COMMON MUD CRABS ON OYSTER BEDS OF DELAWARE BAY

by
John J. McDermott and Franklin B. Flower
Oyster Research Laboratory
Bivalve, New Jersey

INTRODUCTION

In the past, various predators and parasites of oysters in Delaware Bay have been studied by members of the New Jersey Oyster Research Laboratory. However, no serious attempt has been made to investigate the distribution and habits of the common mud crabs (Family - Xanthidae) which are so prevalent and probably contribute to the overall oyster mortality on the New Jersey beds in Delaware Bay.

During field trips throughout the bay we have noticed in various areas, dead oysters with the valves still held intact by the ligaments and chipped to various degrees along the margins. This damage was attributed to crabs, but it was uncertain as to whether this damage was caused by blue crabs (Callinectes), mud crabs or other crabs present in the bay.

In June we began to accumulate information concerning the various species of mud crabs in Delaware Bay with the hope of learning something about their distribution, their capabilities as oyster predators, to observe whether or not there is any detectable difference between the method of blue crab and mud crab destruction and to add to our knowledge of the life history of the various species, since adequate information for this area is lacking.

Over 2200 mud crabs have been collected during June and July from various areas on the natural and leased oyster grounds on the New Jersey side of the Delaware Bay. A satisfactory quantitative method of sampling has not yet been devised. All collections were obtained by means of large oyster dredges during our routine oyster sampling. Crabs were picked at random from the contents of the dredge hauls. All mud crabs collected have been identified as to species and sex, ovigerous females noted and length and width measurements were obtained. It should be emphasized that the data following are for the months of June and July of 1952 and only indications of mud crab distribution can be realized.

DISTRIBUTION

Five species of mud crabs have been found in the area investigated (from Arnold's Point to the Cape May Canal). Four of the five species have been previously reported from New Jersey waters. To our knowledge, Hexapanopeus angustifrous (Benedict and Rathbun) has not been reported.

Rhithropanopeus harrisii (Gouldi), the "brackish water mud crab" is the dominant species in the upper part of the natural seed beds where the salinity averages about 5 o/oo, and is common with other species along the shallow littoral regions of the bay. This species has not been found in the central areas or deepwater portions of the bay. As yet we do not know whether salinity, topography or other factors determine its distribution. This is the smallest of the five species.

According to the data available Hexapanopeus angustifrous seems to have a clearly marked distribution. It is found only in the southern portion of the bay proper and is the least common of the five species since only 20 individuals

have been collected. It is a much more common species south of New Jersey.

Eurypanopeus depressus (Smith) the "flat mud crab" has been found in practically all of the areas investigated. It is scarce, however, in the comparatively fresh water areas up the river where Rhithropanopeus is abundant, and is also scarce in the middle of the bay. Its distribution is similar to that of Rhithropanopeus, as it is limited somewhat to the littoral areas.

The most common species of mud crab in Delaware Bay is Neopanope texana sayi (Smith), the "southern mud crab". It is scarce along the littoral areas of the bay and in regions of low salinity. It is expecially abundant on the oyster beds of the bay proper where the "red beard sponge", Microciona prolifera Verrill is found. Large numbers of these crabs are found within this species of sponge.

Panopeus herbstii Milne Edwards, the "large mud crab" is the largest of the five species, many being over 30 mm. in width. It is found three-quarters of the way up the natural beds, but is not common in this northern limit. It is most common in the bay proper where it and Neopanope are the dominant species.

The following is our information on ovigerous females of the various species from all areas for the months of June and July:

Species	June Collections		July Collections	
	No. of females	Per cent Ovigerous	No. of females	Per cent Ovigerous
Rhithropanopeus	61	62	187	24
Eurypanopeus	157	29	152	58
Neopanope	191	39	259	63
Panopeus	92	20	93	35
Hexapanopeus			6	100

From these data it can be seen that while the majority of the females of Rhithropanopeus were ovigerous in June, the majority of the females of the other four species were ovigerous in July. The average water temperature for June was 75°F while for July it was approximately 80°F during the early part of the month and 85°F during the last two weeks of this month. Water temperature may be a factor which influences the depositing of eggs of the various species in this bay.

DAMAGE TO OYSTERS

Only a few laboratory experiments have been undertaken to determine the extent and type of damage inflicted on oysters by mud crabs.

In one experiment, 20 experimental oysters ranging in size from 1.7 to 7.1 cm. in length were placed in an aerated tank with 5 Panopeus herbstii (range in width 28.0 to 35.5 mm.). In 13 days when the experiment was terminated, a total of 14 oysters had been destroyed. These ranged in length from 1.7 to 5.4 cm. The 6 remaining oysters ranged in size from 4.1 to 7.1 cm. The 18 control oysters were all healthy at the end of the experiment. Smaller oysters were destroyed first by these crabs. Six small oysters ranging in length from 1.8 to 3.7 cm. were placed in the same tank with 3 of the same mud crabs and the remaining

6 larger oysters from the proceeding experiment. Within 36 hours 3 of these
small oysters were destroyed while the larger oysters remained unharmed. These
and other small-scale experiments have indicated that the "large mud crabs"
(under laboratory conditions) are selective in their feeding on oysters, since
they quickly destroy the small thinner-shelled oysters before attacking the larger
ones.

In other experiments it was found that both Panopeus and Neopanope will feed
on the "ivory barnacle" Balanus eburneus Gould. A shell containing approximately
300 barnacles (this years set) and 6 oysters ranging in length from 1.8 to 5.5 cm.
were placed in a tank containing 6 Neopanope (average width 15 mm.). In 7 days
none of the oysters were destroyed whereas all but 20 of the barnacles were eaten.
These same oysters were then put into a tank with 2 large Panopeus along with two
shells containing approximately 200 barnacles each. Within 12 hours the two
smallest oysters (1.8 and 2.3 cm.) were crushed and eaten while the barnacles were
not harmed. In three more days 2 more oysters (3.4 and 4.7 cm.) were destroyed
along with 1/2 of the 400 barnacles.

Although both Panopeus and Neopanope can easily crush and devour barnacles
it does not seem likely that these are their principal food especially in the bay
proper where barnacles are scarce and these two species are so abundant. On the
northern two-thirds of the natural oyster beds, however, where there are large
sets of these barnacles, they may be a principal source of food for mud crabs.
What appeared to be mud crab damage to barnacles has been noted when examining
dredge hauls from this upper bay region.

Panopeus will chip the margins of the valves of oysters until it can get at
the soft parts. The right valve is usually chipped more extensively as seen in the
laboratory and in the field. In chipping 5 cm. oysters, a large Panopeus will
grasp the margin of the valves with one or two chelipeds and force the movable
fingers downward on the right valve so that the crab's body rises off the sub-
stratum with only the tips of the walking legs remaining on the surface. This
action produces the most force on the right valve, therefore the observed greater
damage to this valve. The right valves of small oysters, 2cm. long or less are
often crushed in half and the body parts devoured.

In the future we intend to carry out field experiments and further laboratory
experiments expecially with this year's oyster set. Only one and two year old
oysters have been used in the above experiments.

<center>MUD CRAB PREDATORS</center>

Probably many species of fish prey on mud crabs but as yet we only have infor-
mation on the feeding habits of two species. Most of the fish examined were ob-
tained in making dredge hauls on the oyster beds.

Opsanus tau (linnaeus) the "toadfish" or "oyster-cracker" is a common inhabi-
tant of the Delaware Bay oyster beds. The contents of the alimentary tract of
21 "toadfish" have been examined. These fish ranged in length from 5 mm. to 32
cm. (total length - from snout to end of caudal fin). Sixteen of the 21 were feed-
ing on mud crabs of various species. Two of the five not feeding on mud crabs
had no food at all; one had consumed a large spider crab Libinia sp.; one had fed
on a young blue crab Callinectes; and the last was a young fish (5 cm. long) which
was feeding on gammarids and one megalope stage crab of unknown species. As many as
8 crabs were found in a single fish. Of the 19 fish in which food was found in

the gut, all were feeding on crustaceans. Sixteen or 84% were feeding on mud crabs.

Neopanope was the dominant species of mud crab found in the toadfish. This is explained by the fact that most of the fish were collected where this crab is very abundant. Two fish caught in the Maurice River were feeding on the "brackish water mud crab" Rhithropanopeus. Eurypanopeus and Panopeus were also found, but very seldom.

Other organisms also found in some toadfish collected were the "small hermit crab" (Pagurus), "sand shrimp" (Crago), "grass shrimp" (Palaemonetes), "tube shrimp" (Unicola), "soft clam (Mya), "razor clam" (Ensis), "salt marsh clam" (Mulinia), and "limpets" (Crepidula).

Only 3 "northern swellfish" Spheroides maculatus (Block and Schneider), ranging in length from 13.6 to 23.1 cm. were examined. All were feeding on young "razor clams", one was also feeding on this years "soft clam" set, and remains of mud crabs were found in one individual.

A "northern swellfish" caught in Raritan Bay in May 1952 has been feeding on at least 61 "mud snails" Nassa obsoleta Say as determined by identifying the opercula of the snails as no shell fragments could be found.

CONTROL OF MUD CRABS

The methods used in combating the oyster drill should prove effective in controlling the mud crab. We have observed that the deck screen is very effective in securing many mud crabs. The drill dredge and drill traps should also be quite effective. The Flower Suction Dredge is a very efficient method of catching these crabs. If all oyster growers would use some of these devices in controlling their drill population they would also be relieving their oyster beds of another predator, the mud crab.

HOW STRONG IS THE OYSTER?

By Paul S. Galtsoff

U. S. Shellfish Laboratory, Fish and Wildlife Service
Woods Hole, Massachusetts

The adductor muscle is the most conspicuous organ in the body of the oyster. Its place of attachment to the shell is marked, in the Atlantic oyster, by the dark pigment which in specimens 4 to 5 years old, covers an area varying from 1.2 to 4.1 square centimeters.

The adductor muscle comprises a very substantial part of the tidal weight of the oyster tissues. In the large oysters, it constitutes from 19 to 25 percent of the body weight. In the smaller oysters, about 6 to 7 centimenters long, the figures are higher, varying from 32 to 42.5 percent. The importance of the adductor muscle in the biology of the oyster does not lie, however, in its relative weight but in its physiology. Particularly significant for the life of the oyster is the ability of the muscle to remain in a contracted state for several days and even weeks. By tightly closing its valves and retaining the sea water in its shell cavity the oyster is able to remain alive for a long time after it has been taken out of water. By keeping the shells hermetically sealed it protects itself against the irritating or poisonous materials that may be temporarily present in the sea water. In the same manner it defends itself against the attacks of starfishes, crabs, small fishes, and other enemies.

In the process of evolution the oyster had lost its foot and is therefore deprived of the power of locomotion. It has no means of attacking other animals, and for its own defense it must rely only on its adductor muscle which keeps the mollusk safe within its calcareous home.

Everyone who has attempted to shuck an oyster knows that considerable force is needed to pry the valves apart. It is, of course, interesting to know how strong is the resistance of the muscle which keeps the valves closed. Before answering this question we shall consider first the force which tends to bring the valves apart and is overcome by the oyster when the shells are closed. As everyone present in this room knows, the two valves of the oyster are joined together by a rubber-like hinge which acts as a spring. As soon as the adductor muscle is relaxed, loses its elasticity, or is cut, the valves gap. We can determine the force needed to counteract the pulling force of the hinge by a very simple method. The oyster body is removed from the shell, care being taken not to injure the hinge. One valve is immobilized on a suitable base, while air pressure is applied to the other valve over the area of the muscle scar. The apparatus can be constructed from a glass syringe the plunger of which rests on the upper valve, and the air is forced by means of a bicycle pump through a nozzle of the syringe. Shell closure is recorded on a kymograph, while the pressure is measured by a U-tube mercury barometer connected by means of a T-tube to glass syringe. With such equipment it was possible to measure the pulling force which the muscle must exert in order to keep the shell tightly closed. The force naturally varies, depending on the size of the oysters and their condition, the range of variations extending from 165 grams in the weak and small specimens to 884 grams in the large and strong oysters. Referred to the area of the muscle scar these figures vary from 83 to 488 grams per square centimeter. I found no correlation between the strength of the hinge and the area of the muscle scar.

Physical properties of the hinge substance change very rapidly when the shells are out of water. To avoid the error due to the changes in the elasticity of the hinge, the preparation should be kept moist all the time. Fortunately, the reading can be accomplished within a few minutes before any changes in the hinge substance may occur.

The determination of the pulling force needed to stretch the adductor muscle was made by using a very simple technique. One valve of the oyster was mounted in a strong mixture of cement and sand with a small addition of Plaster of Paris. The upper valve was attached by means of a metal hook, mounted in a cement cap, to the lower surface of the left pan of a laboratory balance. The weights were placed on the right pan, and shell movement was recorded on a kymograph. The oyster was kept either in the air or in running sea water. This method has considerable advantages over the old ones used first by Plateau in 1884 and repeated with slight modifications by Marceau in 1909 and Tamura Tadashi in 1929 and 1931. In the previous methods the edge of the shell was bored through to insert a rod or a hook to which the weights were attached. My observations show, however, that the presence of a foreign body inside the shell cavity irritates the tissues and that under such conditions the adductor muscle changes its tonus more rapidly than when it is not disturbed by mechanical stimuli. Furthermore, by applying the pulling force directly over the area of the attachment of the muscle one can measure the force used without the computation needed in case the weights are attached to the edge of the shell.

The results of determinations made on oysters from Narragansett Bay, Peconi. Bay, and Cotuit show that under the force of 10 kilograms (22 pounds) the muscle stretches immediately, and the shell gaps. Complete fatigue, evidenced by the inability of the muscle to contract in response to electric shock or to mechanical or chemical stimuli, occurs within a relatively short time, varying from 15 minutes to 1 hour. Many oysters are unable to withstand this force, and their muscles break under the pulling force varying between 5 to 10 kilograms (11 to 22 pounds).

The strength of the adductor muscle is dependent on the general condition of the organism. As a rule, oysters kept for about 2 months in laboratory tanks become weak, and their muscles are easily stretched. Likewise, after spawning and during the period of reabsorption of gonads the muscles frequently break under a pulling force of only 4 kilograms.

French and Japanese investigators mentioned above paid a great deal of attention to the maximum force needed to break the muscle. In the present investigation an attempt was made to study the time required to produce complete fatigue of the muscle without inflicting irreparable injury or rupture of the muscle. The results show that there exists a relationship between the pulling force applied to the adductor muscle and time required for its complete fatigue. This can be seen from the following data of the experiments in which the oysters were kept in running sea water:

Force applied (in kilograms)	Time needed for complete fatigue (in hours)
1	409
2	44 to 71
4	7 to 24
5	6 to 10
6	5 to 28
7	6
10	$\frac{1}{4}$ to 1

Individual variations are very large, and many more observations are needed in order to arrive at an equation representing this relationship. But the general trend is obvious.

Similar results were obtained with the oysters kept in the air, the only difference being that the latter experiments could not be carried on for more than 48 hours since longer exposure resulted in the drying of tissues and death.

One can notice from the above data that time required to produce complete fatigue rapidly decreases when the pulling force reaches and exceeds 6 kilograms. The data referring to the lighter weights are, however, very interesting from a biological point of view since it is quite probable that the pulling forces of 1 or 2 kilograms may be experienced by the oyster in nature when the mollusk is attacked by star fish. It seems almost incredible that a small organism whose body weight varies from 10 to 15 grams without the shell is capable of continually lifting and holding the weight of 1 kilogram for more than 17 days. Experiments which are now under way at Woods Hole show that this remarkable performance is due to the so-called locking or catch mechanism of the muscle the existence of which was postulated long ago by famous physiologist, I. Pavlov.

In locking its muscle at a definite tonus level the oyster does not need an additional supply of energy derived from respiration. Under this condition its oxygen intake does not increase and often drops very conspicuously. This has been demonstrated by the experiments on oyster metabolism which are now being conducted at Woods Hole. Whether the oyster utilizes some energy resulting from glycolysis and other chemical changes in the adductor muscle may be disclosed by future research. From a biological point of view it is clear, however, that the physiology of the adductor muscle of the oyster provides a very effective protective device which greatly contributes to the survival of the oyster in its natural environment.

ON FOOD AND FEEDING OF LARVAE OF THE AMERICAN OYSTER, C. VIRGINICA

Harry C. Davis
U. S. Fish and Wildlife Service,
Milford, Connecticut

Larvae of our American oyster, Crassostrea virginica, are limited, a. to the types of food they can utilize, by factors other than the size of the macro-organism or particle. We have previously reported feeding experiments using four species of marine bacteria, three species of flagellates, detritus, and organic media (Davis, 1950). In those experiments neither the bacteria nor the flagellates had any effect on the rate of growth of oyster larvae. In one such experiment, for example, in cultures that received sulfur bacteria as food, the larvae averaged only 9?.0? µ in length at 14 days, a size which was not significantly different from that of larvae in unfed control cultures. In a pair of parallel cultures, fed a mixed culture of phytoplankton consisting chiefly of Chlorella, the larvae averaged 140.75 µ in length by the 14th day.

The same series of experiments also showed that detritus, which we had collected from several sources, was not utilized by the larvae, and that, at low concentrations, organic media added to the larval cultures caused no increase in the rate of growth of the larvae. At higher concentrations of the organic media, the larvae were killed by the dense bacterial growth that resulted from the organic enrichment of the sea water.

On the basis of the above experiments it was reported that our mass culture of green phytoplankton consisting chiefly of Chlorella, which we shall refer to as "mixed Chlorella", was the best food for oyster larvae then available. It did not consistently give good growth, when fed to larvae during their earlier stages, but once the larvae had attained a size of approximately 125 µ they appeared to utilize the mixed Chlorella quite readily.

More recently we have tested, as food for oyster larvae, nine additional species of marine bacteria, isolated from the mud of Milford Harbor by Dr. Burkholder of Yale University, and six species of flagellates, obtained from Dr. Russell of the Plymouth Laboratory, England. One culture fed our mixed Chlorella, one culture fed B. coli plus a bacteriophage and one unfed control culture completed the experimental series (Fig. 1).

The larvae in all ten cultures receiving bacteria and those in the culture receiving flagellate F, and unidentified chrysomonad, grew less rapidly than did those in the unfed control culture. Moreover, in the ten cultures receiving bacteria, the larvae were all dead by the 11th day. We can assume that these bacteria and flagellate F were not utilized.

In the remaining six cultures that received supplemental food, the larvae grew more rapidly than did those in the unfed control. We can conclude therefore, that oyster larvae probably do utilize the five species of flagellates, Dicrateria inornata, Chromulina pleiades, Hemiselmis rufescens, Isochrysis galbana and Pyramimonas grossii, as well as our mixed Chlorella.

A direct comparison of the values of the different flagellates, as foods for oyster larvae, is not possible from this experiment. Unfortunately, due to difficulties in mass culturing the flagellates, we were
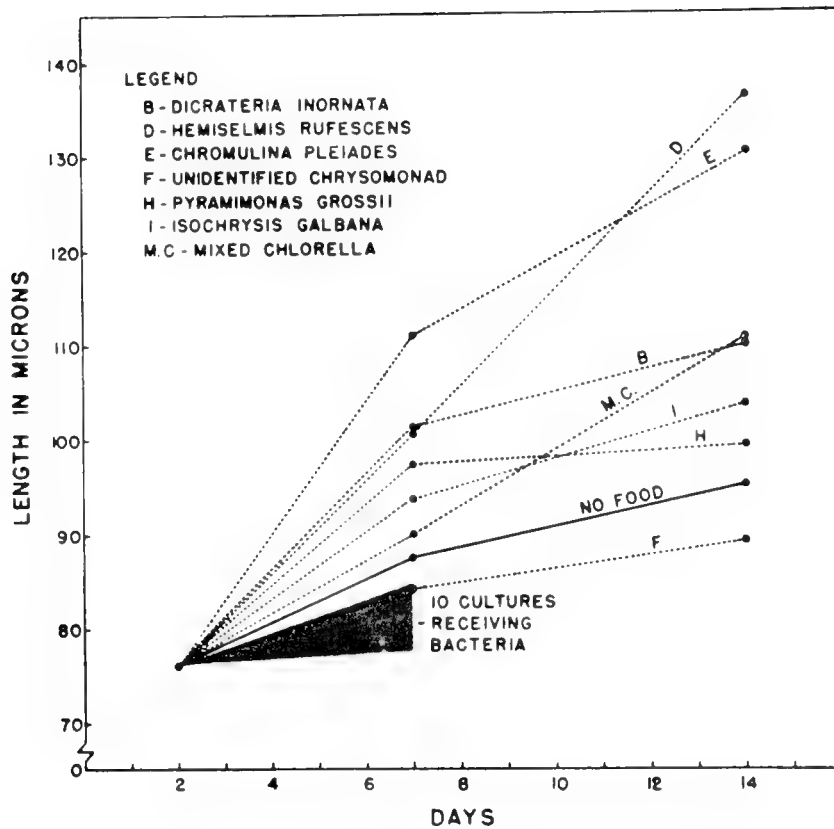
FIG. 1. Growth of larvae of C. virginica fed different foods. Each
point on a curve represents the mean length of 100 larvae.
Flagellates D. E. and I were fed at the rate of 10,000 per
ml/day. Flagellates H and F were fed at the rate of 5,000
per ml/day. Flagellate B was fed at the rate of 10,000 per
ml/day for first seven days and 5,000 per ml/day thereafter.

not able to feed equal numbers of each flagellate nor were we able to keep the rate of feeding constant in the culture receiving flagellate B (Dicrateria). In this culture Dicrateria was added at the rate of 10,000 cells per ml/day for the first seven days but after that it was necessary to reduce the rate to 5,000 cells per ml/day.

Flagellate I (Isochrysis) and flagellate D (Hemiselmis) were added at the rate of 10,000 cells per ml/day throughout the experiment, while flagellates F (the unidentified chrysomonad) and H (Pyramimonas) were added at the rate of 5,000 cells per ml/day. Moreover, since this was a preliminary experiment single cultures were used and the results are consequently less reliable than in later experiments where duplicate or triplicate cultures were used to test each food. Isochrysis, for example, appears to be a comparatively much poorer food here than it proved to be in later experiments. Note that the curves for mixed Chlorella and flagellate D (Hemiselmis) break slightly upward after the seventh day while all the others break slightly downward (Fig. 1). This may indicate that these two forms are more rapidly utilized by the larvae in the later stages of development.

Subsequently, in experiments of factorial design, we have fed oyster larvae various combinations of those flagellates which the previous experiment had shown the larvae could utilize. We hoped to estimate the relative value of the different flagellates, as foods for oyster larvae, and to determine whether some combination of them might provide a more balanced diet which would result in more rapid larval growth.

The results of a typical experiment of this kind show that certain combinations did appear to give more rapid growth of larvae than others (Fig. 2). The combination of the flagellates B ≠ E, for example, gave more rapid growth than either the combination B≠ D or E ≠ D. Nevertheless, the greatest differences are correlated with the total number of flagellates given per day. Thus the three combinations B ≠ I, D ≠ I, or E ≠ I, each consisting of a total of 15,000 flagellates per ml/day gave definitely more rapid growth than did the combination B ≠ E ≠ D ≠ I which consisted of a total of only 12,500 cells per ml/day. Moreover this latter combination in turn gave slightly more rapid growth than did any of these combinations consisting of only 10,000 flagellates per ml/day. Thus, the total number of flagellates added appears to be the most important factor and the implication is that the different species of flagellates are of nearly equal value as foods for oyster larvae.

To estimate the relative value of the various flagellates more accurately we have computed the growth curves that we would expect to get if oyster larvae were fed each of the flagellates alone at the rate of 10,000 cells per ml/day (Fig. 3). Using the data from the previous experiment the growth increment due to each flagellate was computed, for each period, by the method of least squares. The points for the curves were then determined by adding the computed growth increment due to each flagellate to the observed mean length of larvae in the unfed control culture.

In this experiment and throughout the series of experiments on these flagellates, with the exception previously noted (Fig. 1), flagellate I (Isochrysis) has given more rapid larval growth, cell for cell, than any of the other flagellates.
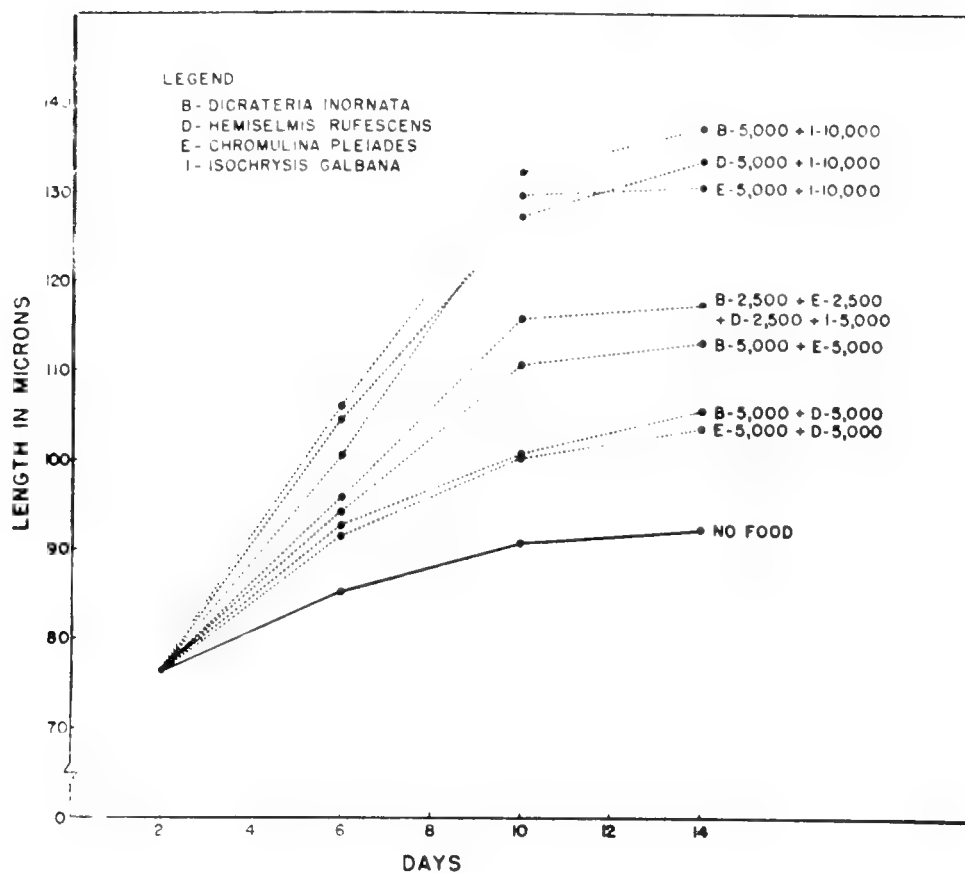
FIG. 2 Growth of larvae of C. virginica fed combinations of flagellates.
Each point on a curve represents the mean length of 100 larvae
from each of duplicate cultures. Numbers following letter de-
signation of flagellates indicate rate of feeding in number of
flagellates per ml/day.

Although the curve for B (Dicrateria) is higher, in this experiment, than the curve for E (Chromulina), in approximately 50 percent of the experiments the reverse is true. It is known that the chemical composition of micro-organisms varies with their physiological condition. It appears probable, therefore, that in these experiments the differences in rate of growth of oyster larvae brought about by variations in the physiological condition of Chromulina and Dicrateria are as great as or greater than differences dependent upon which of these two species the oyster larvae are fed. Flagellate D (Hemiselmis) appears to be the poorest food of all (Fig. 3), although here as in Figure 2 the slope of the curve between the tenth and 14th days may indicate that Hemiselmis is more readily utilized by older larvae. Unfortunately this flagellate culture was lost and we have no further data on its effect on the rate of growth of oyster larvae.

Bruce, Knight & Parke (1940), in feeding experiments on larvae of the European oyster, O. edulis, rated flagellate I as a "good to very good" food while rating flagellate B as "fair". Although their tabulated data seem to indicate that flagellate F was utilized and that flagellate D was not, the authors do not discuss these two flagellates further.

The second part of the problem was to determine whether, by combining the various flagellates, we could provide a diet that would bring about more rapid growth of oyster larvae than could be obtained by feeding an equivalent number of cells of a single species. From a statistical viewpoint, if, by totalling the calculated separate growth increments due to the different foods, we obtain a satisfactory fit to the growth increment observed when these foods are used in combination, we will have no reason to believe that there are interactions. In other words, if we can add the growth increment due to B and the growth increment due to E and obtain a total that agrees closely with the growth increment observed when B and E were fed in combination, we will have no reason to believe that the combination B $\neq$ E is any better as a food than equivalent quantities of either B or E alone.

A statistical analysis of the results of the previous experiment shows no evidence that any of the combinations, of flagellates tested, provides a more balanced diet, or results in more rapid growth of oyster larvae, than would equivalent amounts of any of the flagellates separately (Table 1). The observed mean lengths of larvae receiving combinations of the different flagellates as foods, were compared with the mean length calculated as the sum of the growth increments due to the separate foods plus the mean length of larvae in the unfed control cultures. At six days the maximum difference is 1.8 μ, at 10 days it is 4.0 μ, and at 14 days it is 4.7 μ. These differences are of the same order of magnitude as differences between parallel cultures receiving the same treatment and it can be shown that the fit between calculated and observed mean lengths is satisfactory, so the foods appear to be completely additive and no interaction is indicated. Bruce, Knight & Parke (1940) believed that a combination of the flagellates H and I were especially suitable as food for larvae of O. edulis but did not test for an interaction. Thus far we have not tested this combination with larvae of C. virginica.

Studies to determine what concentration, of any single species, of these flagellates, would be required to produce maximum growth of oyster larvae are still in progress. Preliminary tests indicate, however, with

TABLE 1. Differences between observed and calculated mean lengths of oyster larvae receiving different foods.

| FOODS	6 DAYS			10 DAYS			14 DAYS		
	Observed	Calculated	Diff.	Observed	Calculated	Diff.	Observed	Calculated	Diff.
No food	85.2	85.2	-	90.8	90.8	-	92.3	92.3	-
B+E	94.2	92.9	1.3	110.8	107.9	2.9	113.3	109.8	3.5
B+D	92.8	93.7	-0.9	100.7	102.2	-1.5	105.7	107.2	-1.5
B+I	106.0	105.5	0.5	132.2	134.2	-2.0	137.2	138.4	-1.2
E+D	91.4	91.1	0.3	100.5	101.4	-0.9	103.8	104.2	-0.4
E+I	100.5	101.7	-1.2	129.7	132.6	-2.9	130.6	133.0	-2.4
D+I	104.6	102.8	1.8	127.2	123.2	4.0	133.5	128.8	4.7
B+E+D+I	95.9	97.4	-1.5	115.8	114.9	0.9	117.3	118.4	-1.1
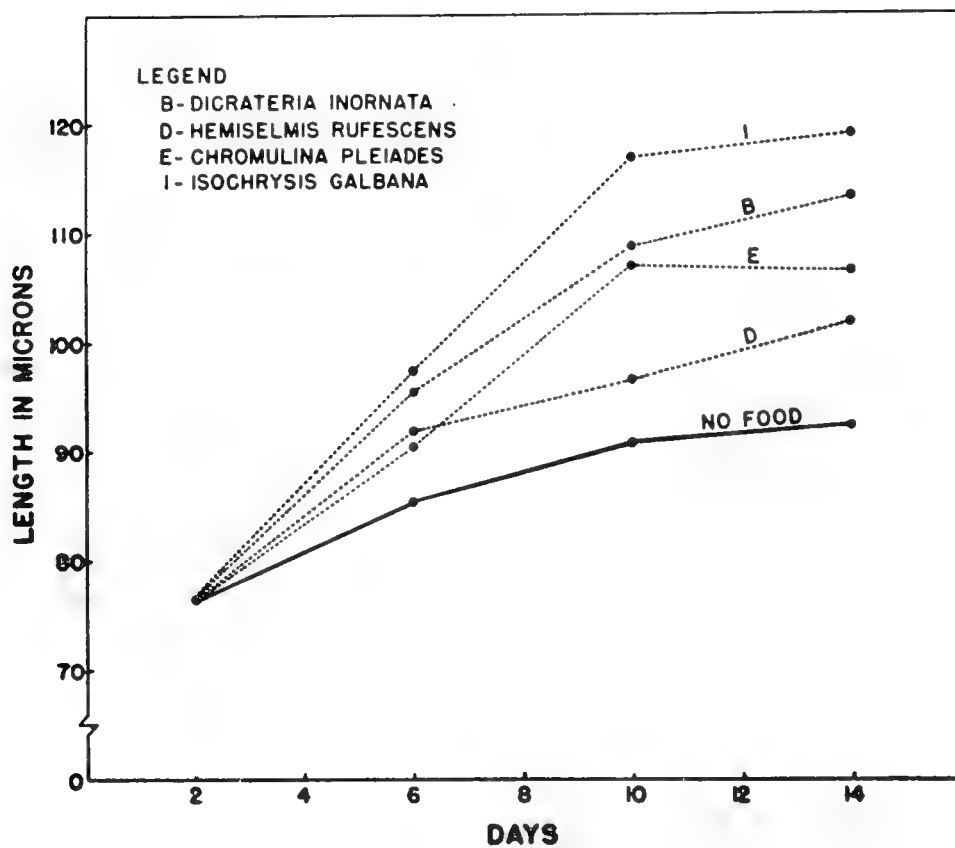
FIG. 3. Growth of larvae of C. virginica showing computed effect of feeding different flagellates at the rate of 10,000 per ml/day. Based on the data in Figure 2.

Dicrateria alone as the supplementary food, that this flagellate must be fed at the rate of 25,000 cells per ml/day (the highest rate tested), or higher, to produce the most rapid growth of larvae possible in cultures such as ours, which contain approximately 5,000 larvae per liter. Isochrysis has not been tested in varying concentrations, but the larvae grew rapidly when this flagellate was fed at the rate of 20,000 cells per ml/day. When Chromulina was used as the supplemental food, however, the larvae grew slightly faster in cultures receiving 15,000 cells per ml/day than in cultures receiving 20,000 cells per ml/day.

Our experiments indicate, therefore, that while the concentration of flagellates required to give the most rapid growth of larvae probably varies with the species of flagellate, none of the flagellates, that were utilizable, gave toxic effects when added to our larval cultures at rates up to 15,000 cells per ml/day and for certain species up to 25,000 cells per ml/day. Korringa (1949), on the other hand, although he obtained good growth of larvae and a heavy spatfall of O. edulis in his 1947 experiments with concentrations of flagellates between 10,000 and 20,000 per ml., concluded from later experiments that "water initially containing more than 5,000 flagellates, or a commensurable great number of other phytoplankton, should be mistrusted as it may contain toxic concentrations of phytoplankton metabolites from the first day the tanks are filled." Imai and Hatanaka (1949) indicate that, in culturing larvae of Crassostrea gigas, they strive to keep the concentration of the flagellate, Monas sp., at a concentration of only 1,000 to 2,000 per ml. in their cultures. These authors have, at maximum, only about 200 larvae per liter, however, and such low concentrations of flagellates would give too small a quantity of food to provide appreciable growth of larvae in cultures such as ours with a concentration of approximately 5,000 larvae per liter.

In other experiments we sought to determine the effect of a pure culture of Chlorella sp. on the rate of growth of oyster larvae. In these experiments Chlorella sp. was tested both alone and in combination with some of the flagellates (Fig. 4). Using triplicate cultures for each treatment, one trio served as a control and received no supplementary food, one trio received Chlorella sp. alone (5,000 per ml/day of each) and one trio received Chlorella sp. (50,000 per ml/day) in addition to the combination of flagellates B $\neq$ E (5,000 per ml/day of each).

Our mixed Chlorella culture, although apparently being more effectively used by larvae in the later stages of development, had been utilized from the start (Fig. 1). With a pure culture of Chlorella, however, in this and in several repetitions of the experiment, the effect on the growth rate of the larvae during the early stages, although small, is consistently negative (Fig. 4). This is true both when pure Chlorella is added alone, and when it is used in combination with the flagellates. Some time between the sixth and 12th days, however, the larvae appear to become able to utilize Chlorella sp. and it accelerates their growth markedly.

Statistical tests similar to those previously mentioned show, however, that there is no evidence of an interaction of Chlorella sp. with the flagellates but that the growth increment of the combination of B $\neq$ E $\neq$ C is the algebraic sum of the growth increments due to B $\neq$ E and to C. These experiments explain our previously published observations (Davis, 1950) that mixed Chlorella, while
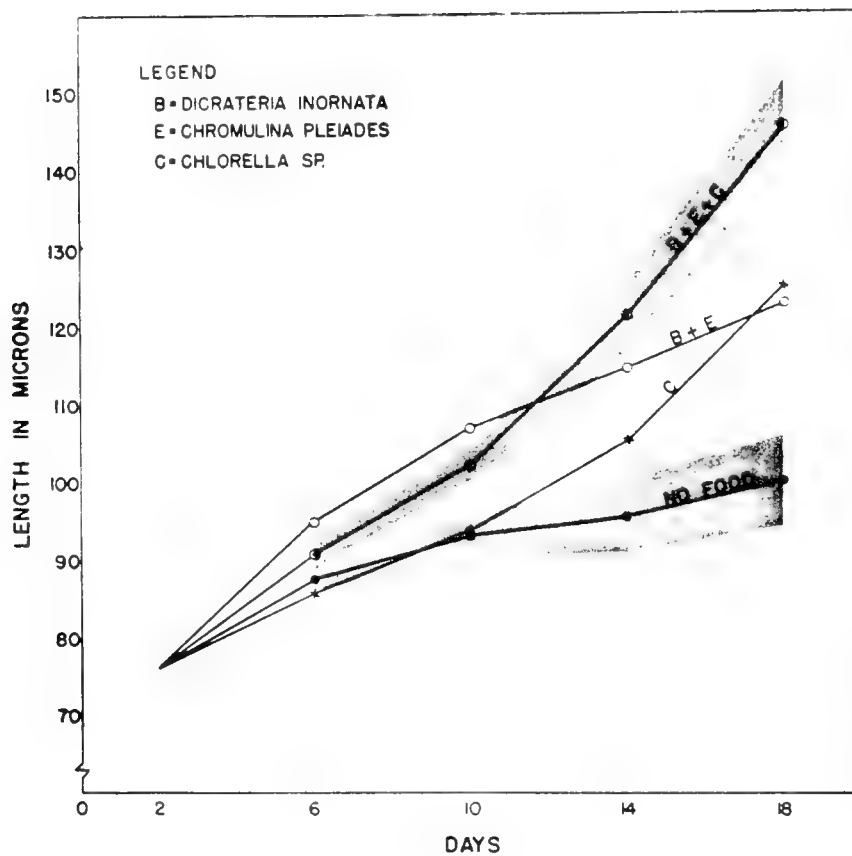
FIG. 4. Growth of larvae of C. virginica fed Chlorella sp. alone and
in combination with flagellates. Each point on a curve re-
presents the mean length of 100 larvae from each of triplicate
cultures. Shaded areas represent 95 percent confidence bands.
Flagellates B and E fed at the rate of 5,000 per ml/day and
Chlorella sp. fed at the rate of 50,000 per ml/day.

not consistently a good food during earlier larval stages, served quite well as food during later larval stages. The good growth obtained during later larval stages is undoubtedly due to the utilization of Chlorella itself by the oyster larvae. The inconsistency of growth of earlier larval stages is understandable since, from these experiments, we know that during these stages the larvae do not utilize Chlorella. Apparently then, growth during the early larval stages, when mixed Chlorella was used as a food, was due to other forms which might or might not be present at any given time in our mass culture of Chlorella, and to small amounts of food in the sea water, which may, as in the experiment shown in Figure 4, carry the larvae through the critical early stages until they can utilize Chlorella.

Cole (1936) after a review of the literature on the European oyster (Ostrea edulis) states that the conclusion that the larvae are able to develop on Chlorella "is not borne out by the results of critical feeding experiments. These all tend to show that the larvae is unable to digest this alga." We have shown here, however, that Chlorella is utilized by older larvae of C. virginica and we have unpublished data showing that larvae of Ostrea lurida can be reared to metamorphosis on either mixed Chlorella or a pure culture of Chlorella. In addition Loosanoff and Davis, 1950, Loosanoff, Miller and Smith, 1951 and Loosanoff and Marak, 1951 have reared O. edulis, Venus mercenaria, Mya arenaria and several other species of lamellibranch larvae using our mixed Chlorella as the chief food. Such results certainly indicate that the ability to utilize Chlorella, as a food, is common to several species of lamellibranch larvae.

In the course of our experiments certain differences in rate of growth of oyster larvae, in different members of a trio or pair of cultures receiving the same treatment, appeared to be due to differences in the number of larvae present in the culture. An experiment, in which triplicate cultures at each of four different concentrations of larvae were used, indicated an inverse relation between the concentration of larvae and their rates of growth (Fig. 5). These cultures all received 50,000 cells per ml/day of our mixed Chlorella as a supplementary food.

The between culture variations, at the two lower concentrations of larvae, were abnormally great, while at the higher concentrations the between culture variations were normal. Thus at 14 days the average size of larvae in the various cultures were as follows:

NUMBER OF LARVAE PER LITER	AVERAGE SIZE Culture No. 1	AVERAGE SIZE Culture No. 2	AVERAGE SIZE Culture No. 3
640	140.0	140.1	125.17
2,785	110.50	111.57	126.55
18,580	102.65	103.35	105.35
32,980	98.35	98.70	101.60

The 95 percent confidence bands (Fig. 5) were calculated with the discordant values included. We are probably justified in concluding that,
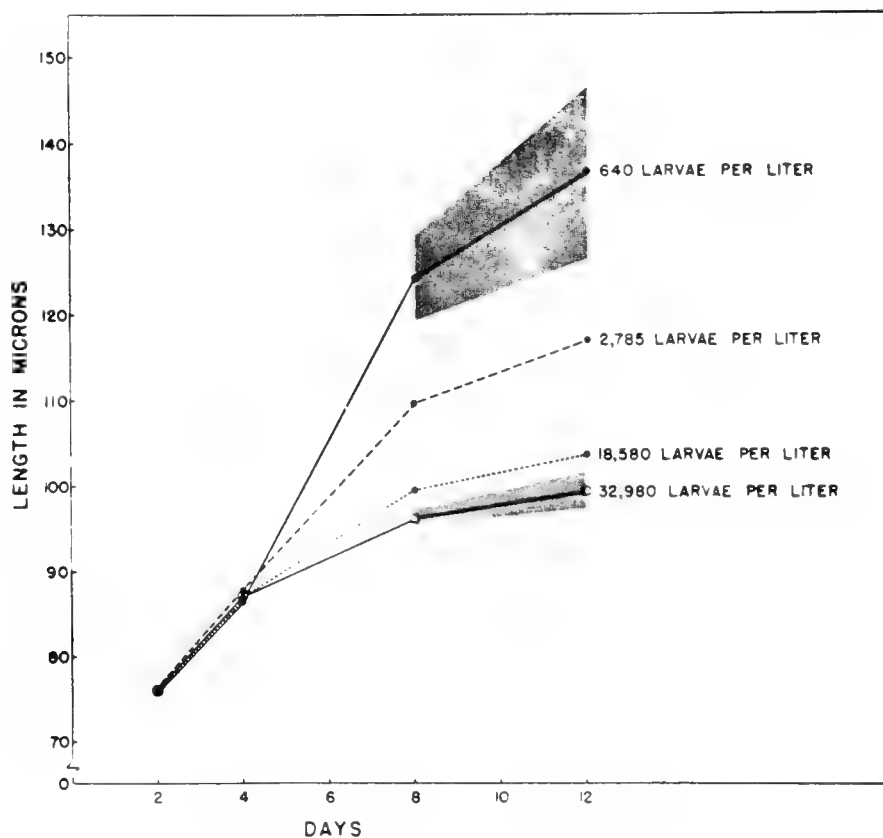
FIG. 5. Growth of larvae of C. virginica cultured at four different concentrations with food constant at 50,000 cells per ml/day. Each point on a curve represents the mean length of 100 larvae from each of triplicate cultures.

with all cultures receiving equal quantities of this food, there is an inverse relation between the concentration of larvae in a culture and their rate of growth, at least after the eighth day. The failure of the inverse relation to appear earlier is probably due to the inability of oyster larvae to utilize the mixed Chlorella readily during the earlier stages of development.

We do not know whether similar results would be obtained with other foods, but suspect that they would be, nor do we know what results would be obtained if the quantity of food were kept proportional to the concentration of larvae. The results of the experiment suggest, however, that the concentration of larvae in a culture must be considered in comparing rates of larval growth.

Other differences, which make comparison of rates of larval growth difficult, are those between successive cultures in which the larvae receive identical treatments. The factor or factors responsible for these differences affect not only the rate of growth of larvae but also, in some experiments, their survival as well. The growth curves of the larvae in the unfed control cultures of seven successive experiments illustrate these differences (Fig. 6). In the experiments of February 18 - March 8 and of February 4-18, the curves appear normal since growth of the larvae in the unfed cultures, although slow, was continuous throughout the 14 days of the experiments. The growth of larvae appears subnormal throughout the May 14-24 experiment when compared to the above curves, and in the experiments of March 12-22 and June 9-19 almost no growth occurred in these unfed cultures. In the experiment of March 25 - April 8 the flattening of the curve between the tenth and 14th days, which reflects an almost complete lack of growth of the larvae during this period, appears abnormal. In the experiments of April 28 - May 8 and May 14 - 24 similar abnormal flattening of the growth curves occurred between the sixth and tenth days.

In each of the three experiments, in which the flattening of the growth curves of the unfed cultures occurred after the sixth day, there were also seven pairs of cultures receiving different foods, yet all cultures showed similar flattening of their growth curves simultaneously.

A pronounced simultaneous upswing of growth curves in all cultures has been noted in one experiment and minor simultaneous upward trends were noted in several others. Such simultaneous changes in the growth curves of the larvae in all cultures of an experiment, regardless of food treatment, must be a reflection of variation in some unknown factor or factors common to all cultures. The factors common to all cultures, and least susceptible to control, are the physical, chemical and microbiological constituents of the sea water in which the larvae were reared.

Also strongly suggestive of a variation, in the physical or chemical constituents of the sea water, was the variation in peak densities of flagellate cultures grown in media prepared from sterilized sea water enriched with constant amounts of nutrient salts. Culture media prepared from sea water taken during periods of poor larval growth give lower peak densities of flagellates than does media made up from sea water taken during periods of good larval growth.
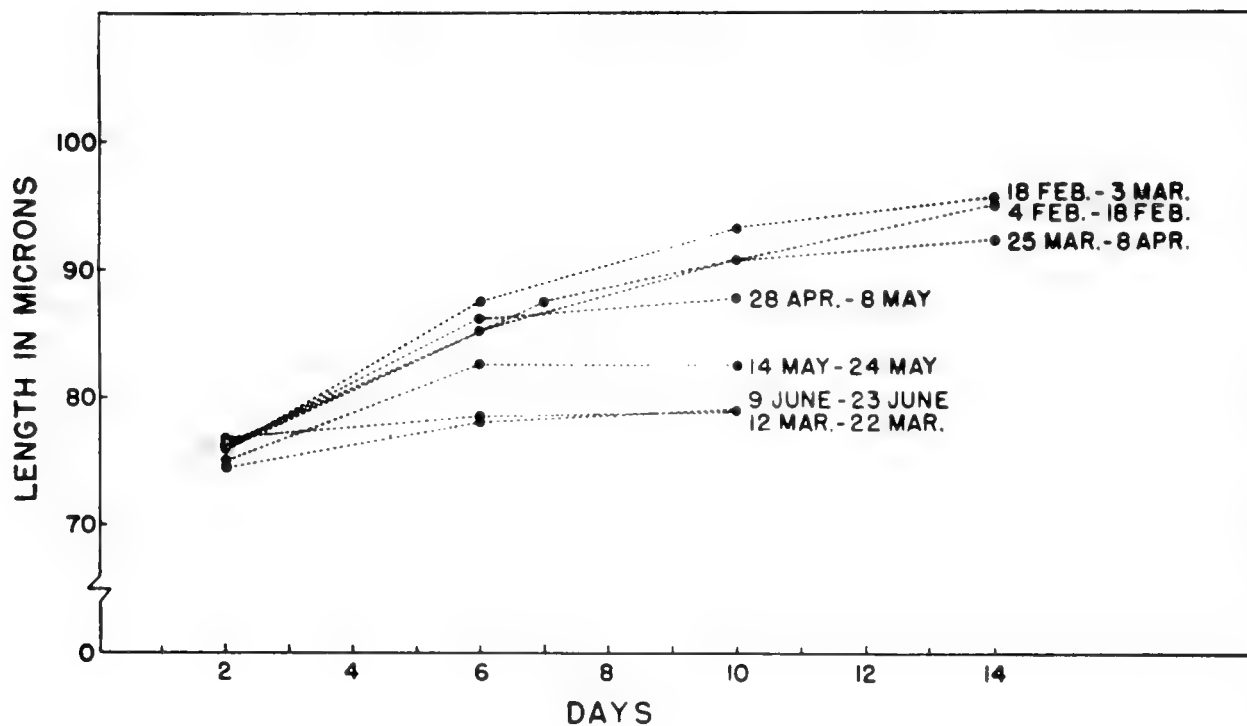
FIG. 6. Growth curves of unfed control cultures of C. virginica larvae showing variations between seven successive experiments. Each point on a curve represents the mean length of 100 larvae from each of duplicate or triplicate cultures.

In all of our experiments, in cultures that received flagellates known to be utilizable, the larvae have grown more rapidly than those in the parallel unfed control cultures. Yet we have not been able to overcome completely the effects of the unknown factor (or factors) by supplemental feeding. For example, under the conditions existing during the May 14-24 and June 9-19 experiments, feeding a combination of the flagellates Isochrysis and Dicrateria produced only about 1/5 as much growth of larvae, in ten days, as did identical concentrations of the same combination of flagellates in the experiment of March 25 - April 8. This suggests that one phase of the action of the unknown factor may be to affect the ability of the larvae to utilize the food that is available.

Several authors have suspected unidentified variations in sea water of affecting their results. Loosanoff, Miller & Smith (1951) noted a lack of uniformity of results in consecutive experiments with larvae of Venus mercenaria and considered it possible "that at different times the water itself contained certain dissolved substances which, in a manner not yet understood, affected the rate of development of bivalve larvae." Wilson (1951), working in England with polychaete larvae, found a difference between sea water from two different areas and attributes the poor growth of larvae in water from one of these sources to a lack of "some unknown constituent, essential for healthy development" of the species of polychaetes he used. One of these unidentified variants may be that described by Collier, Ray and Magnitzky (1950) who reported a substance in sea water, that can be measured photometrically with N-ethyl carbazole, the concentration of which could be correlated with the pumping rate of oysters.

Wangersky (1952) reported that the substance, measured photometrically with N-ethyl carbazole, was a mixture of dehydroascorbic acid and a rhamnoside. He concludes "that the vitamin is present in the sea largely in the form of dehydroascorbic acid." Although we have not yet tried dehydroascorbic acid, we have added ascorbic acid to the sea water in which the larvae were reared. Such additions did not improve the rate of growth of oyster larvae; they merely resulted in a dense growth of bacteria which killed the larvae.

To verify that it was not deleterious changes in the sea water in our laboratory system causing the poor growth and high mortalities of our June cultures, an experiment was designed to compare the laboratory sea water with sea water taken directly from Milford Harbor in enamel buckets. Parallel cultures of oyster larvae were started, two in each type of sea water. In addition a single culture of larvae of Venus mercenaria was started in laboratory sea water at the same time. All culture received approximately 50,000 cells per ml/day of the mixed Chlorella as food.

At 14 days all the oyster larvae in both types of sea water were dead, while the Venus larvae still appeared quite healthy and were growing normally. At 18 days the Venus larvae had reached setting size and a count revealed that approximately 55 percent of the larvae, that had been counted 12 days earlier while still in the straight hinge stage, were still living. Thus, although growth was somewhat slower than average (Loosanoff and Davis, 1950; Loosanoff, Miller and Smith, 1951) the Venus larvae had lived and grown to setting stage, as in several previous experiments (Loosanoff, 1950), under conditions in which the oyster larvae had all died. We agree, therefore, with Wilson (1951), who concluded "it is evident that many animals find no difficulty in living and reproducing under water conditions that seem to affect some other species adversely."

We do not yet know what this "water condition" is, nor whether it is the presence of some inhibitory or toxic substances that causes poor growth, or as Wilson (1951) believes, it is the lack of something necessary for good growth. Our experiments do indicate that one phase of its action is to affect the ability of larvae of C. virginica to utilize the food that is present.

SUMMARY

1. None of the 13 species of marine bacteria, tested to date, was utilized as food by oyster larvae.

2. Five species of flagellates, Dicrateria inornata, Chromulina pleiades, Isochrysis galbana, Hemisalmis rufescens and Pyramimonas grossii, were utilized as food by oyster larvae, while another, an unclassified chrysomonad, in addition to the three flagellates previously reported, was not.

3. Chlorella sp. was not utilized as food by young oyster larvae but was utilized during later larval stages.

4. None of the combinations of foods tried gave any evidence of providing a more balanced diet or more rapid larval growth than could be obtained by feeding equivalent quantities of a single food. The effects of all foods tested, including Chlorella, are additive.

5. When equal numbers of cells are fed, different species of flagellates give different rates of growth of oyster larvae.

6. Species of flagellates also differ in the number of cells needed to give maximum growth of oyster larvae.

7. The maximum concentration of food organisms that can be created in water containing oyster larvae, without unfavorably affecting the larvae, varies with the species.

8. With the number of food organisms equal, the rate of growth of oyster larvae had an inverse relation to the number of larvae per unit volume.

9. Variations between rates of growth of larvae in cultures receiving the same treatment in successive experiments appear due to some variable factor in the sea water that affects the ability of the larvae to utilize the food that is present.

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PRELIMINARY STUDIES ON THE FIELD CULTURE, BEHAVIOR, AND TRAPPING OF THE LARVAE OF THE HARD CLAM, VENUS (=MERCENARIA) MERCENARIA L.

Melbourne R. Carriker,
Zoology Department, Rutgers University

In New Jersey the present annual catch of 5 million pounds of hard clams (Fish & Wildlife Service, 1950) is harvested from a wild crop. If the availability of seed could be enhanced, enemies effectually controlled, nutritional and other limiting environmental factors improved, and faster growing meatier clams bred, this crop could be augmented many fold. The speaker has been engaged since 1947 in research directed at increasing the availability of seed. This has involved field and laboratory research, principally in Little Egg Harbor, on the little known late swimming and early bottom dwelling stages. Present sets are generally scattered, slow, and undependable. As more is learned about the behavior of young clams it may prove practical to interpose aids in the early stages in order to swell the productivity of our native populations.

The principal emphasis in the research of the summer of 1951 was focused on a study of the setting behavior of the larvae in a field laboratory. It was hoped these observations would suggest methods for their capture and study in native waters. Accordingly, data on the climatology and hydrology of the Little Egg Harbor area was collected; food for the nourishment of the larvae was cultured clam larvae were grown to post-setting stages; the temporal distribution of clam larvae in local waters was followed; and preliminary methods for the trapping of native clam larvae in the field were developed.

The choice of Little Egg Harbor for these studies was a fortunate one. Safe harborage for the station, which consists of a floating laboratory, cabin annex, and motorboat, is available in a tidal creek at close proximity to the bay. A large population of native hard clams covers the shallow bottom of the 30 sq. mile estuary. Salinity of the water is remarkably uniform both vertically and horizontally, ranging during the summer between 28-31 °/oo. The mean range of the tide measures 2.2 feet, and non-tidal drift is negligible. Thus clam larvae during their one week of planktonic existence drift back and forth in a nutrient pool of well mixed water the greater volume of which never reaches the ocean.

Loosanoff and his colleagues (Loosanoff and Davis, 1950; Loosanoff et al., 1951) have been eminently successful in rearing hard clam larvae in their laboratory by feeding them food cultures of mixed micro-organisms grown in aerated sea water enriched with 1 gram of commercial fertilizer per liter of sea water. These food cultures, however, would not sustain our larvae beyond a length of 150 μ. Spurred by Pomeroy's (1951) recent discovery that inorganic phosphate and calcium ions are absorbed by the gills of oysters, we increased the concentration of fertilizer ten fold. Best results in growing larvae were obtained by feeding them a Chlorella-innoculated culture heavily fertilized (10 g./l.) with 6-3-6 organic fertilizer alkalieed with marble chips. This food was beneficial to the larvae only after the micro-organisms had passed through a natural succession of 3 to 4 weeks, during the course of which they displayed many color changes. Both filtrate (using Whatman filter paper No. 42) and whole culture were nutritious. Raising larvae on the filtrate of these cultures is significant. Larger forms such as Chlorella may be tentatively eliminated from the diet and attention focused on the possible role in larval nutrition of minute organisms such as bacteria and of organic and inorganic non-protoplasmic substances.

In the absence of compressed air for the aeration of larval cultures in the field, we improvised a plunger jar apparatus patterned after the Browne plunger

jar of English investigators (Browne, 1897)). This proved highly satisfactory.
It was possible to maintain a maximum of 12 fifteen liter jars in the apparatus
and to operate it automatically for 48 hours at a time. The general arrange-
ment and operation of the apparatus is as follows: Every second day at high
tide creek water was pumped manually from the motor boat through a fire hose in-
to 4 barrels arranged in series on a platform outside the culture cabin. From
this elevated reservoir the water flowed slowly through 1/4 inch tubing into an
automatic plunger bucket. As this bucket filled and emptied, completing the
cycle approximately once every 40 seconds, it oscillated a large balanced frame
mounted over the culture jars. Slightly tilted glass plates immersed in the
culture jars and suspended from the eaves of the plunger frame thus rose and fell
with each oscillation of the frame and gently circulated the contents of the jars.

Fertilized clam ova for these studies were procured from artificial spawn-
ings during the summer - - - but with difficulty. Over 1,000 clams were handled
in the spawning pans and only 50 spawned. Larval cultures were manipulated as
described by Loosanoff and Davis (1950). By the collective use of the plunger
apparatus and the food cultures described we raised 4 separated larval broods
(about one million clams) to post setting size. Setting occurred in about 10 days,
a time only slightly longer than that observed for native larvae. Sudden drops
in culture temperatures to 15.8°C. during cool August nights had no apparent lethal
effect on either pre-or post-setting clams.

Since the growth of larvae in mass cultures does not facilitate the study
of single larvae, we developed a running water micro-culture dish for this pur-
pose. 1 cc. watch crystals were collared with plankton silk and connected by fine
siphons to a large dish. Screened fresh bay water and food culture were added
daily. With occasional brushing and pipetting out of detritus from the micro-
dishes it was possible to retain the plankton silk open for about 10 days. Much
use was made of individual larvae in the micro-cultures in observations on sett-
ing behavior and attachment preferences.

A wide variety of substrates was employed in both the micro and the mass
cultures; clean smooth glass and bivalve shells; clean deeply Cliona-pitted bivalve
shells; paraffin surfaces sprinkled with fine and coarse sand; deeply pitted
leached slag; fine sediments composed of minute fecal pellets and plant and ani-
mal fragments; and varying depths of sands of assorted particle size.

The behavior of hard clam larvae changes markedly as the larvae grow older.
Swimming stages or veligers (approximately 98-166 μ long) remain rather uniform-
ly distributed in the water. Older swimming stages develop a foot. This is
extended as they attempt to contact substratum. Once contact is effected the
larvae move searchingly over the substratum, then suddenly extend the velum and
swim off to a different site, alight, and crawl further. This searching behavior
is undoubtedly useful to them in locating favorable setting surfaces, and is dis-
played for several days. The name pedoveligers (166-218 μ) is suggested for this
stage. During the searching period the byssus gland in the foot is developing
rapidly and the clam commences weak byssal attachment to the substratum. Soon
the velum is lost and the clam alternates for a considerable period of time be-
tween byssal attachment and crawling. Detachment from the byssus thread is a rap-
id and complete operation. The term "setting" as applied to hard clams is here
used synonymously with byssal attachment. The term crawler (200-304 μ) is pro-
posed for this stage which alternates between crawling and byssal attachment.
Eventually the byssus gland in the crawler becomes functionless and the young clam
or juvenile maintains its position in the bottom by means of its foot alone.

The behavior of setting larvae is most instructive. They seem to prefer
affixing the byssus thread to a solid surface covered by a thin layer of sedi-
ment and then to remain suspended in the sediment above. Consistently the densest
sets took place over surfaces, whether smooth or pitted, coated by thin accu-
mulations of detritus. Several observations suggested that crawlers possess a
strong response to contact (thigmokinetic). For example, with no observed ex-
ception those crawling in clean micro-dishes attempted with untiring persistence
to dig into the glass with the distal tip of the foot. When fine sediment was
sprinkled near the crawlers they continued moving vigorously and at random until
covered. When completely buried they ceased all locomotory activity and attached.
A second example: some 50,000 crawlers (260 to 500 μ in length) were placed in a
clean finger bowl in shallow water. In about an hour the majority had aggregated
in grape-like clusters and attached to the glass. Thus in the absence of suitable
sources of contact stimulation they aggregated and gained contact with each other.
Additional observations demonstrated that crawlers in the open react strongly to
light, moving actively as soon as cool light is directed upon them.

In addition to the information gained on the setting behavior of young clams
in the laboratory, knowledge has accrued in earlier field work which was applied
in the construction of traps for the capture of native clam larvae. These field
observations suggested that the larvae are influenced in the selection of the
setting site by differential water currents such as are created by depressions in
the substratum, by obstacles in the path of the flow, and by variations in the
topography of the bottom such as are found along the submerged banks of channels.
Field studies have also demonstrated something of the extensive destruction of
newly set clams by predators and the need for protecting young set. The perennial
difficulty in the study of newly set clams in the field is the detection and se-
gregation of the seed from the fine sediments in which they set. Thus it is fur-
ther desirable that traps attract and hold veligers in significant numbers and
provide for ease of detection and manipulation of the seed. On the basis of these
qualifications numerous types of traps were devised but the bottle-top type has
proven the most successful to date.

This type was constructed of the upper third of a narrow-mouth one gallon
glass bottle 6 inches in diameter at the open end. A large paraffined nail in
a one hole rubber cork was inserted in the neck of the top, and dowelling 8 inches
long inserted snugly into the neck of the top. Two or 3 shells were placed around
the dowelling. Plastic screening with openings 1 square mm. in size was wrapped
over the dowelling and the bottle top and secured at the neck with plastic twine.
The screen on some of the tops was coated with copper paint to minimize fouling;
this did not seem to affect the setting of the larvae within. Traps were housed
on a special frame which made possible the placement of the traps in the shallower
portions of Little Egg Harbor. The frame consisted of a long stout anchor pole
driven deeply into the bottom and a second slender pole to which the traps were
attached on cross pieces. In setting the traps we guided the slender pole down
the anchor pole, pressed it slightly into the bottom and tied it at the top to the
anchor pole. When the traps were tended the slender pole was gently raised into
the boat.

Most of the traps were placed in the middle of Little Egg Harbor in water
about 2.2 meters deep at low water. A total of 483 hard clam seed were obtained
in 5 traps during the month of August. The heaviest set in any one trap was 202
seed. The preliminary success of these traps may be attributed to the presence of
a relatively still pocket of water under a baffle, the accumulation of fine layers
of sediment within the trap, and the elimination of larger predators by the screen.
The steep upper sides of the bottle top may aid in retaining crawlers within

the container, and the shallowly sloping lower contours and cultch provide zones at the "shores" of the accumulating pool of sediment thin enough to attract the crawlers.

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Setting of Oyster Larvae and Survival of Spat in the St. Mary's
River, Maryland, in Relation to Fouling of Cultch.

J. H. Manning
Chesapeake Biological Laboratory
Solomons, Maryland

The State of Maryland has planted in recent years an annual average
of over 800,000 bushels of oyster shells, and the seed production program
is currently being expanded.

It is generally recognized that cultch planted any considerable time
before the season of oyster spawning will catch fewer spat than if planted
shortly before setting occurs. However, limitations on equipment and storage
facilities, the weather, and the great area of Chesapeake Bay and its tri-
butaries combine to extend Maryland's shell planting program over a relatively
long period. Thus it is of some importance to know, for a particular seed
area, how far in advance of the expected time of spatfall cultch may be planted
without material loss in spat-catching qualities. The only experimental evi-
dence bearing upon this question was reported at the 1950 meeting of the Nat-
ional Shellfisheries Association by Sieling. The experiment here reported
was preliminary to an investigation which, it is hoped, will contribute to
the more efficient use of cultch in Maryland's seed areas.

On May 1, June 1, and June 26, 1951, 12 wire bags each containing 25
scrubbed oyster shells were suspended beneath the laboratory pier at Solomons
Island, on the Patuxent River, where a heavy early May barnacle set usually
occurs and where there is little if any oyster strike in late spring or early
summer. On July 2 all of these bags were taken up and five shells were re-
moved from each bag for subsequent examination. The bags were immersed in
tubs of river water and transported by truck to the St. Mary's River, where
with an additional 32 bags of scrubbed shells, they were suspended beneath
the pier at Seminary Point. Temperature and salinity differences between
Solomons Island and Seminary Point on July 2 were $1.1^{\circ}C$ and 0.6 parts per
thousand, respectively.

At Seminary Point the State maintains a large shell planting which pro-
duces some of Maryland's test seed, and the Chesapeake Biological Laboratory
has for many years exposed a series of bags of test shells for approximately
weekly periods during late spring and summer months. Records indicate that
the peak of spatfall usually occurs about the second week in July.

Thus on July 2, 1951, there were at Seminary Point a large commercial
planting of shells, bags of test shells of the weekly-exposure series, and
from this experiment, four groups of shells - 12 bags of shells with 63 days'
prior exposure, 12 bags of shells with 32 days prior exposure, 12 bags of shells
with six days' prior exposure, and 32 bags of scrubbed shells not previously
exposed.

On July 18, August 3, and November 9 four bags from each group of shells
were taken up and ten shells removed from each bag. In addition, on August
20 and September 10, four bags of the shells exposed on July 2 were sampled.
All shells were measured to obtain an estimate of the area of their inside
faces, and examined under a binocular microscope. Counts were made of spat,
barnacles, and Bryozoa colonies on shells of the weekly series and on shells

of the sampling of July 2. In all other samplings spat and barnacles were
counted. Counts were adjusted to a basis of organisms or colonies per 500
square centimeters. Very limited numbers of fouling organisms other than
barnacles and Bryozoa were observed. Of these, Sabellid worms and Folliculinids were most numerous, but neither was present in numbers great enough
to merit consideration as a serious deterrent to the setting of oyster larvae.

Figure 1 indicates the extent of barnacle fouling on the several groups
of shells. In each sampling the barnacle population on the shells exposed
May 1 exceeded that of any group of shells put down at a later date, and
the difference in each case is statistically significant at the five percent
level.

FIG. I - BARNACLE COUNTS ON OYSTER SHELLS, ST. MARY'S RIVER, 1951

Figure 2 shows the extent of Bryozoa .... fouling on the four categorized shells as of .... of all on the .... water .... In .... shells .... shared Bryozoa populations significantly higher .... of the other shells. The difference between the colony counts on shells .... from May 1 and those exposed June 26 falls considerably short of statistical significance at the five per cent level.

FIG. 2 - COUNTS OF BRYOZOA
ON OYSTER SHELLS,
ST. MARY'S RIVER,
1951

▲ — EXPOSED JULY 2
● " JUNE 26
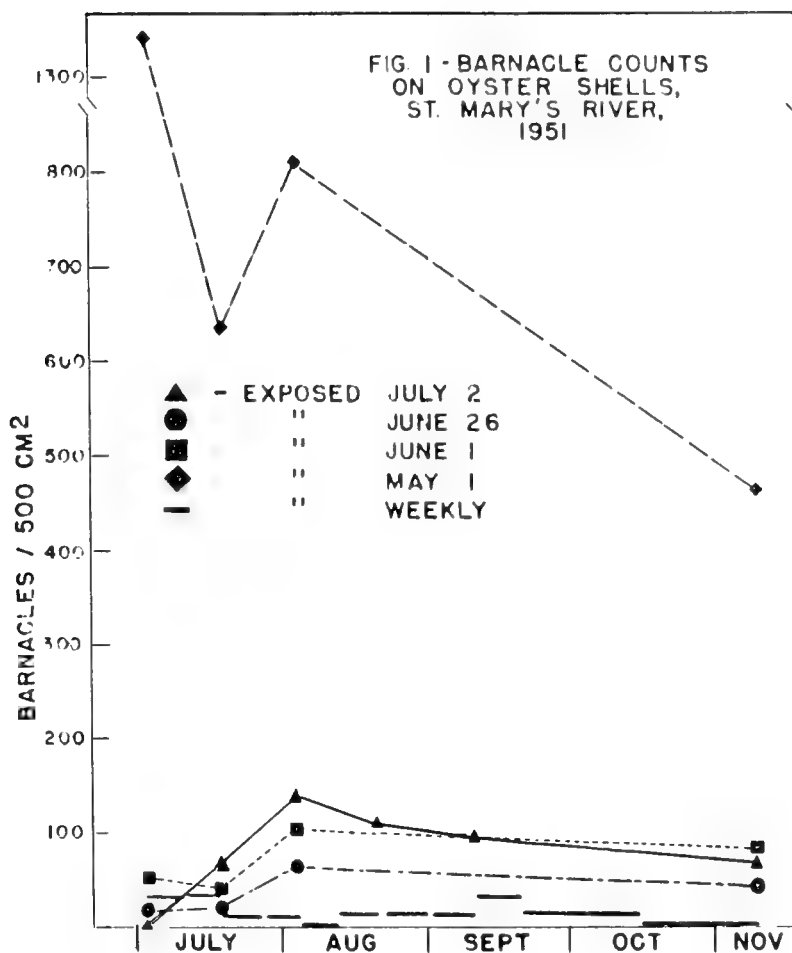■ " JUNE 1
◆ " MAY 1
▬ " WEEKLY

Figure 3 indicates the numbers of spat on the several groups of
shells. No setting occurred before June 20, and only a very light set
up to July 3, after which the rate climbed steadily to a peak in late
July and dropped off abruptly after August 3. Setting was negligible in
the Patuxent River before July 2; only two spat were found on 100 shells
examined. The shells exposed May 1 - the group which caught the signifi-
cantly larger number of barnacles - show a consistently smaller population
of spat than any of the other groups of shells. The differences are
statistically significant at the five per cent level in all but one case -
the July 13 comparison with shells exposed June 1. Among the groups of
shells exposed June 1, June 26, and July 2 the differences in spat popu-
lations are of inconsistent sign and fall short, in seven of nine compari-
sons, of significance at the five per cent level.

FIG.3 - SPAT COUNTS
ON OYSTER SHELLS,
ST. MARY'S RIVER,
1951

▲ - EXPOSED JULY 2
●   "   JUNE 26
■   "   JUNE 1
◆   "   MAY 1
—   "   WEEKLY
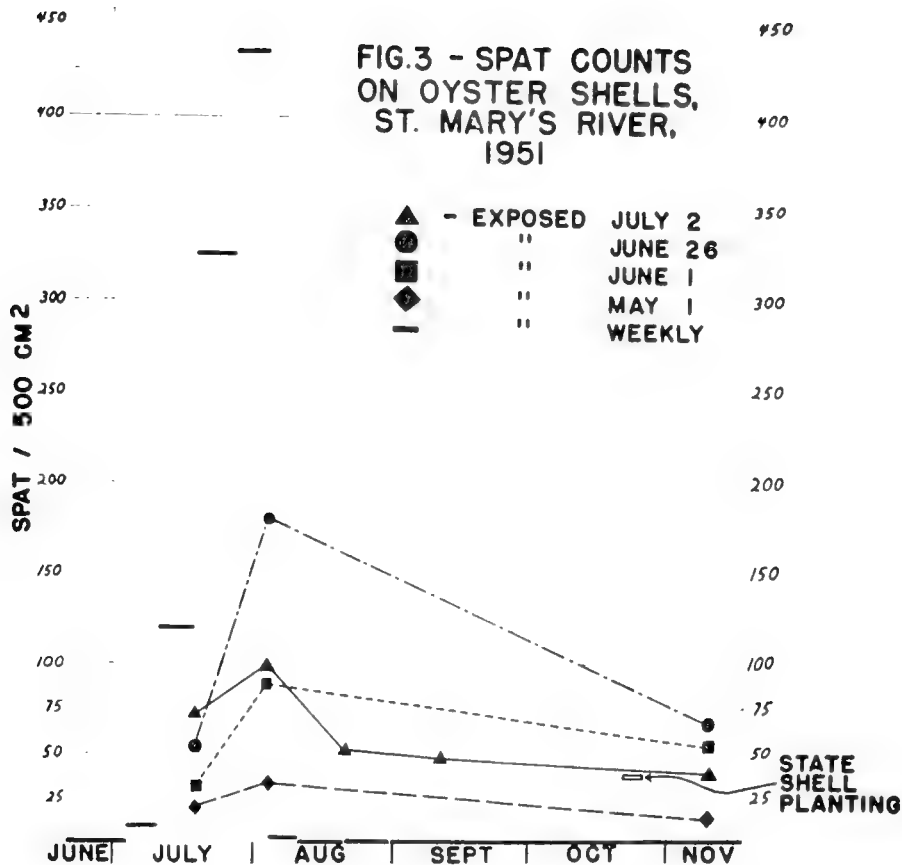
Pairing the spat counts on July 18, August 3, and November 9 with the counts of Bryozoa colonies on shells of corresponding groups on July 2, correlation coefficients of -0.289, -0.084, and 0.268 were calculated. The influence of Bryozoa fouling on the setting of oyster spat appears to have been negligible in this experiment. However, none of the four groups of shells was heavily fouled with Bryozoa during the period of spatfall.

Values of the coefficients of correlation of spat counts on July 18, August 3, and November 9 with barnacle counts on July 2 are -0.579, -0.573, and -0.663. Values of -0.539, -0.597, and -0.669 result from calculation of correlations of spat counts with barnacle counts on the same bags of shells in the July 18, August 3, and November 9 samplings. The stated values of the coefficients of correlation of spat with barnacle populations exceed the five percent level of significance in all cases. A null hypothesis concerning the relationship of barnacle fouling with the setting of oyster larvae may be rejected with considerable confidence.

Since the setting of oyster larvae on test shells exposed for weekly periods was neglibible after August 3, it is assumed that recruitment to the spat populations of the four groups of shells used in the experiment was also negligible during the period August 3 - November 9. Among the groups of shells exposed July 2, June 26, June 1, and May 1, percentages of survival after approximately three months were 39, 37, 62, and 38 respectively. There is nothing in the data to indicate relationship between survival rate of spat and either interspecific or intraspecific competition.

The conditions of this experiment were to some degree artificial. There is some evidence, however, that suspension of the shells in wire bags did not greatly influence the set or survival of spat. Sampling on October 23 of the State shell planting at Seminary Point which was completed during the last week in June, showed a spat population closely comparable to that on shells exposed experimentally on July 2 and sampled November 9.

SUMMARY

1. In this experiment, barnacle fouling apparently inhibited the setting of oyster larvae to the extent that heavily fouled cultch caught only about 25% as many spat as did cultch which was relatively free of barnacles during the period of spatfall. There is no evidence that barnacles had any measurable influence on the survival of spat.

2. Light to moderate Bryozoa fouling had no apparent influence on the setting of oyster larvae or the survival of spat.

Studies on the Setting Intensity of Oysters in Bogue Sound, North Carolina

A. F. Chestnut and William E. Fahy
University of North Carolina
Institute of Fisheries Research
Morehead City, North Carolina

Considerable time and effort have been expended over the past 35 years in this and other oyster producing countries on studies of the setting behavior of oyster larvae. Most of the literature has been critically reviewed and valuable contributions to the knowledge of the behavior of oyster larvae and their setting have been made by Korringa (1940). Recent studies by Cole and Knight-Jones (1949) have shown interesting observations on the gregarious tendency exhibited by larvae of Ostrea edulis.

In following the setting of oysters in a given area variations in intensity of setting are generally found from year to year and even from day to day. Frequently these variations can be correlated with changes in salinity, temperature and other enviromental factors.

Since June, 1948 routine studies have been made in Bogue Sound, North Carolina, on the setting intensities of oysters at the Institute of Fisheries Research. The studies were made along the north shore of Bogue Sound about five miles west of Baufort Inlet. When possible records were kept of the intensity of daily setting. From 1949 through 1950, duplicate series of four clam (Venus) shells in wire baskets were exposed as spat collectors. One series was suspended from the pier. The shells were varied in position for different studies. In 1951, only one station was maintained along the beach and the number of shells as spat collectors was increased to ten. The spat counts were limited to the number of oysters setting on the inner surfaces of the shells. Each of the studies are discussed under the respective headings.

Seasonal variations in setting intensity:

The setting data for 1948 are not complete, but continuous setting was recorded from June 7 through October 7. The heaviest setting was during the week of June 7 to 14 when 12 spat per shell were recorded. In 1949, setting of oysters began on May 16 and continued until the last week of November. The peak of setting occurred during the week of June 20 when average of 35 spat per shell was found. Setting was light through the remainder of the summer, averaging from one to three spat per shell. Heavy rainfall occurred through the summer months. Nine inches of rain was recorded for the week of May 30 to June 6 and six inches during the week of June 30 to July 7. From August 11 through September 22 the rainfall averaged 3.2 inches a week.

During 1950 the first oyster spat was found on May 22 and the peak of setting was during the week of June 5 to 13. The setting intensity was light, averaging 11 spat per shell. A second peak of setting occurred from July 31 to August 7.

The first oyster set in 1951 was found on May 29. During the summer months three peaks of setting were recorded. The heaviest set was during the two week period of June 26 to July 10 when 51 and 52 spat per shell were recorded. A second peak of setting took place from August 22 to 29 and a third peak during October 15 to 22.

The data for 1952 through July show that the first set of oysters was on May 22.
A heavy setting took place the first week of June when the spat counts showed an
average of 458 spat per shell.

From the hydrographic data collected in the area, local rainfall appears
to exert the greatest influence upon setting. (see figure 1) Setting intensity
generally decreased following a two or three inch rainfall. Setting was heavy
during 1951 and the early part of 1952 when rainfall was slight in comparison
to 1948, 1949, and 1950.

The salinity in Bogue Sound remained fairly stable during the four summers,
ranging from 28 to 35 parts per thousand, except when influenced by unusually
heavy rainfall. In 1949, a six inch rainfall lowered the salinity in one week from
28.0 to 22.0 p.p.t. and in 1950 a six inch rainfall lowered the salinity from
32.4 to 20.8 p.p.t. In both cases the salinity increased within two weeks to fall
within the normal range.

Water temperatures generally reach an average of $22.0^{\circ}C$ by the first week
of May and continue to rise to 29° or $30^{\circ}C$ by the first week of July. Tempera-
tures remain above $25^{\circ}C$ from mid-June until the middle of September and then fall
below $20^{\circ} C$ in the middle of October.

Setting intensity in relation to position of shells:

Each basket of shells placed overboard during 1948, 1949, and 1950 was varied
in position in relation to the tidal currents. At the Institute beach one basket
was placed with the shells facing perpendicular to the tidal flow and the other
basket with the inner faces of the shells facing up and down was parallel to the
current. These results are presented in Table II.

At the Institute beach where the shells were exposed to direct sunlight, the
heaviest set was found on the shells facing down.

The baskets of shells suspended from the pier were also placed parallel and
perpendicular to the tidal flow. Spat counts on shells just under the surface
of the water, in the shade of the pier, and at the bottom in a depth of 18 feet
showed only a slight difference between the spat counts on the inner faces of the
shells facing up and down.

The shells placed paralled to the tidal flow in both localities had more
spat attached than the shells placed perpendicular to the current. These results
are included in Table II.

Vertical distribution of setting:

Series of shells were suspended at foot intervals from the high water mark
to one foot above the bottom to determine the variation in setting intensity in re-
lation to depth. Small baskets with four shells each were wired to a length of
chain suspended from the pier and anchored with a cement block. The results for
two weekly studies are shown in Table III. No oyster spat were found attached to
shells at the high water mark. Below low water level, the intensity of setting
showed a trend of increase in number of spat on the shells nearer the bottom.
The adult oysters in this locality are generally limited in distribution to the
intertidal zone.

Intensity of setting on "aged" shells:

During 1951 duplicate series of ten shells were placed overboard in the intertidal zone at the Institute beach. One series was changed daily and the duplicate series was changed at weekly intervals. Spat counts show that the shells changed at weekly intervals had more spat attached than the cumulative total of spat on the shells changed daily for the same period of time. These results are found in Table IV.

The studies were then followed by comparing the number of spat attached to shells which had been soaked or "cured" in sea water for a number of days prior to exposure as spat collectors. Shells were soaked in boiled sea water and compared to shells soaked in untreated sea water at three day intervals up to twelve days. The heaviest setting was found on the shells soaked in untreated sea water for 9 and 12 days. The shells soaked in boiled sea water showed little difference between the number of spat attached to the experimental shells and the controls. These results are in Table V. Shells kept immersed in untreated sea water for long intervals of time from 15 to 25 days, generally become fouled and their efficiency as spat collectors was greatly reduced.

Cole and Knight-Jones (1949) reported a gregarious tendency exhibited by oyster larvae of Ostrea edulis. This was tested at the Institute beach to determine whether oyster larvae of Crassostrea virginica show the same tendency. A group of ten shells were placed overboard for twenty-four hours to collect some oyster spat. The oyster spat were removed from five shells and those on the duplicate set of five shells were circled with a pencil mark. The duplicate sets of shells were then placed overboard and examined after twenty-four hour intervals, for three successive days. The shells that had spat previously attached contained more additional spat than the shells from which the spat were removed each day. Both series of shells did contain more spat than the routine daily spat collectors maintained over the same period. These results are found in Table VI.

Discussion:

Considerable variation in intensity of oyster setting was found during four seasons in Bogue Sound, North Carolina. In some cases the setting intensity seems to be more closely correlated with rainfall than with salinity or water temperature. When precipitation was greater than two or three inches during one week, lower spat counts were noted in the following week. Excessive rainfall did result in a temporary lowering of salinity in some extreme cases.

The setting of oysters was found to be heavier on the under side of shells that were exposed to the direct sunlight, but in shaded areas there was little difference between the number of spat on the under side and upper surface. These results are in accord with the findings of some investigators. Nelson (1927) found that setting of Crassostrea virginica was ten times greater on the underside than on the surface. Hopkins (1937) showed that Ostrea lurida set more heavily on the under side of glass plates and Schaefer (1937) found the same to be true with Crassostrea gigas. Cole and Knight-Jones (1939) found that Ostrea edulis set with greater intensity on the under side of slate and shell collectors, but with glass plates the heavier set was on the upper side. Korringa (1940) found more Ostrea edulis oysters setting on the upper surfaces than on the under side.

Shells placed parallel to the current had more spat attached than shells that were placed perpendicular to the current. These results are in accord with the

findings of Hopkins (1937) for Ostrea lurida, Schaefer (1937) with Crassostrea gigas and Korringa (1940) with Ostrea edulis.

Studies on the vertical distribution of setting of oysters in the past has shown considerable variation in results. Galtsoff and Luce (1930) found in Georgia, that the heaviest setting occurred above the low water level. Loosanoff (1932) and Mackin (1946) both found that the zone of heaviest setting at Wachapreague, Virginia was above low water, but Loosanoff found that heavier sets occurred near the bottom in the James and Corrotoman rivers. MacDougall (1943) showed that heavier sets of oysters were below water at Beaufort, North Carolina. The results in Bogue Sound presented here have shown that more oysters set below low water than in the intertidal zone although few if any adult oysters are found growing below low water. The predatory activities of oyster drills as well as the effects of smothering and crowding by barnacles, tunicates, sponges and other sessile marine organisms must be taken into consideration in such areas as Bogue Sound.

The studies of Cole and Knight-Jones (1949) have shown a most interesting observation on the gregarious tendency of oyster larvae and the discovery that fouled cultch is particularly favorable for the attachment of oyster larvae. The studies in Bogue Sound tend to support the findings of Cole and Knight-Jones for Crassostrea virginica. Although sufficient studies were not made, some evidence is presented that shells soaked in natural sea water have more spat attached than shells that have not been treated or shells soaked in boiled sea water. The results suggest that a film on the shells is important in rendering them more favorable as cultch. The nature of the film which is evident, has not been satisfactorily determined.

Cole and Knight-Jones (1949) suggest from their observations that accurate predictions of spat fall might not be necessary since larvae prefer cultch that is partly fouled but they are careful to point out that in practice this is not true. The constant deposition of silt in estuarine waters is a factor to be considered. The results presented here have shown that in experimental studies immersion of shells for periods of 2 or 3 weeks reduces the efficiency of the shells as spat collectors.

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Table I

Comparison of oyster setting with hydrographical conditions at Institute beach, Bogue Sound, N.C. for 1951

| DATE	TOTAL SPAT/ TOTAL SHELLS	RAIN (INCHES)	S °/oo	TEMP. (°C)
1951				
May 1-7		2.11		22.0
7-15		.04		20.4
15-22		0.85		
22-29	0/12	0	32.3	24.7
29- 5	3/24	0.46	33.3	22.8
June 5-12	149/16	.07	33.8	24.7
12-19	103/20	0.47	34.5	25.5
19-26	180/20	0.17	35.1	30.2
26- 3	2530/50	0.42	35.7	28.8
July 3-10	2129/40	0	36.7	29.2
10-17	988/50	0.78	35.6	29.4
17-24	563/50	0.83	34.2	28.9
24-31	427/70	4.57	32.7	29.4
31- 7	26/70	0.81	33.6	29.4
Aug. 7-15	35/70	0.21	33.0	30.2
15-22	174/70	0.34	34.0	31.5
22-29	1495/50	0.55	34.6	27.0
29- 5	311/70	0.39	33.3	30.7
Sept. 5-12	61/70	0.18	34.5	29.1
12-19	46/50	1.32	33.7	27.3
19-25	42/80	T		
25- 1	2/10	1.86		19.0
Oct. 1- 8	48/10	1.00	31.5	23.7
8-15	329/20	1.52	30.0	19.5
15-22	722/20	.17	28.4	21.4
22-27	31/20	.03	28.0	21.5
27- 5	1/10	1.44	29.6	20.2
5-19		3.48	28.0	14.4
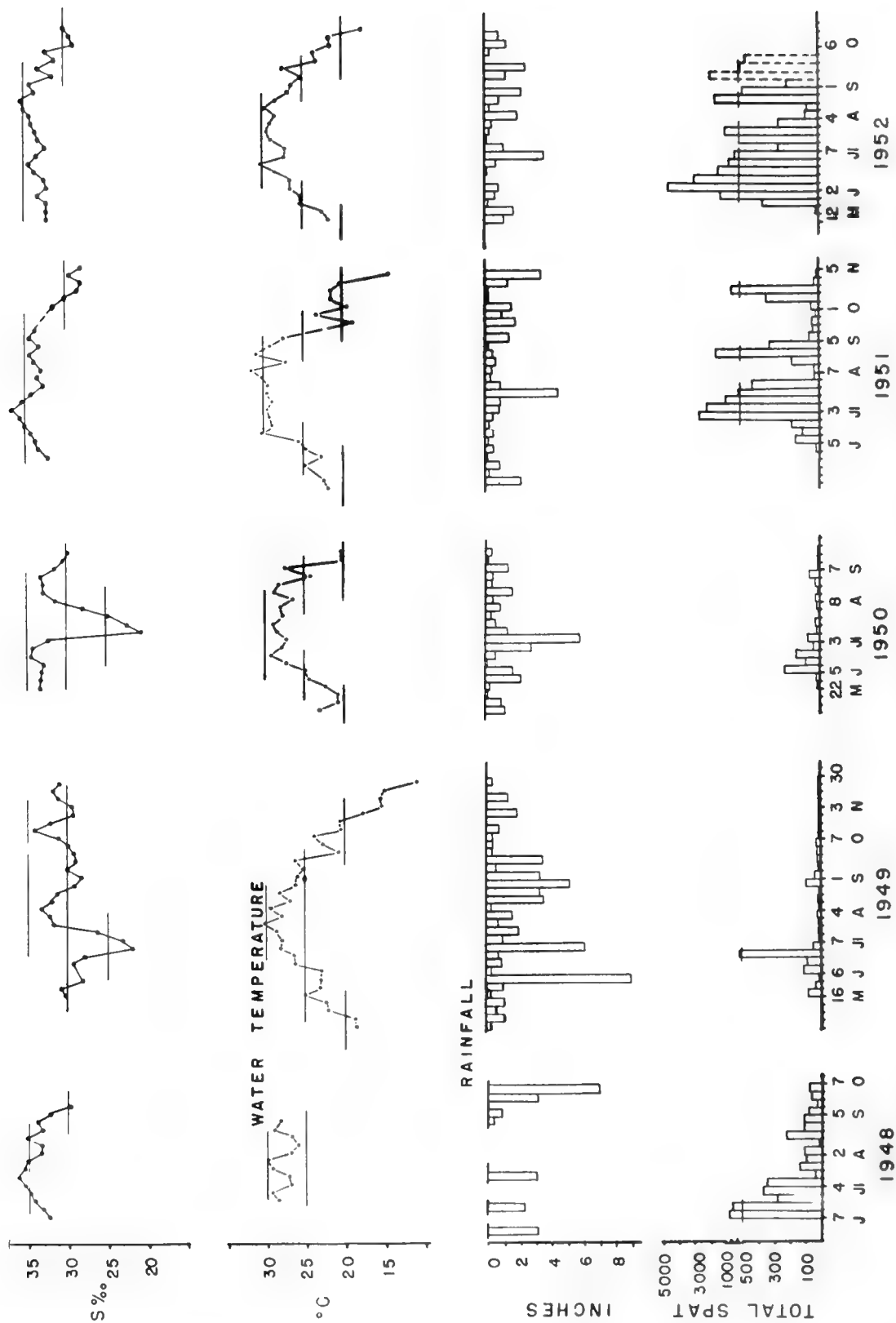
Figure 1. - Comparison of Total weekly oyster set with rainfall, water temperature and salinity at Institute beach, Bogue Sound, N.C., for 1948 - 1952.

Table II

Comparsion of oyster setting on shells facing in different directions at the
Institute beach and Institute pier, Bogue Sound, N.C.

Institute Beach

1948	Upper Surface	Lower Surface
June	266	635
July	38	121
August	61	105
September	9	33
Total	374	894

Institute Pier

1948	Surface		Bottom (18 ft.)	
	Upper Surface	Lower Surface	Upper Surface	Lower Surface
June	161	131	88	143
July	37	33	89	71
August	57	46	73	50
September	—	—	—	—
Total	255	210	250	264

Institute Pier (Surface)

1950	Parallel	Perpendicular
May	12	1
June	496	352
July	142	66
August	67	37
September	60	41
Total	777	497

Table III

Vertical distribution of oyster setting at Institute pier, Bogue Sound, N.C.

Depth	Total Spat	
	5/23-30	5/30-/6/6
2	0	0
1	2	5
L.W.	13	72
-1	19	60
-2-	12	67
-3	14	42
-4-	29	85
-5	28	96
-6	54	108
-7	66	133
-8	46	175
-9	24	169
-10	97	191
-11	87	106
-12	118	152
-13	88	246
-14	101	174
-15	91	170

Table IV

Comparison of Setting Intensity of Oyster Spat on Shells Changed Weekly and Daily,

Institute Beach, Bogue Sound

Date (1951)	Weekly Total	Cumulative Daily Total
5/22 - 29	15	0
5/29 - 6/5	15	8
6/5 - 12	633	373
6/12 - 19	553	258
6/19 - 26	640	450
6/26 - 7/3	2834	2530
7/3 - 10	2829	2129
7/10 - 17	1071	988
7/17 - 24	691	563
7/24 - 31	916	427
7/31 - 8/7	26	20
8/7 - 15	52	35
8/15 - 22	278	194
8/22 - 29	1842	1495
8/29 - 9/5	471	311
9/5 - 12	87	61
9/12 - 19	73	46
9/19 - 25	81	42

Table V

Number of Spat Setting on Shells Soaked in Boiled and Natural Sea Water

Boiled Sea Water

12 da.	9	6	3	0
6/23-24	18	16	23	16	26
6/24-25	13	10	6	7	13
6/25-26	29	32	17	13	32
6/26-27	15	20	5	6	4
6/27-28	13	11	11	9	8
TOTAL	88	89	62	51	83

Natural Sea Water

12 da.	9	6	3	0
7/19-20	56	29	28	33	19
7/20-21	35	34	45	30	14
7/21-22	35	23	11	13	22
7/22-23	22	22	18	13	13
7/23-24	9	1	7	5	2
TOTAL	157	109	109	94	70

Oyster Set on Shells Soaked in Natural Sea Water

5 da.	3	1	0
7/30-31	22	21	11	10

25 da.	20	15	5	0
8/26-27	89	90	103	132	114

14 da.	11	9	7	4	0
9/13-14	9	17	13	13	10	10

Table VI

Numbers of larvae setting on experimental shells with spat and spat removed compared to daily setting intensity at Institute beach, Bogue Sound, N. C.*

Date	Spat Left (46)	Spat Removed	Daily Set
7/22-23	69	56	53
7/23-24	32	24	8
7/24-25	207	191	47
Total	308	271	108

* Total set on five shells in each group

Some Observations on Rate of Growth of Oysters in the Maryland Area

G. Francis Beaven
Maryland Department of Research and Education
Solomons, Maryland

The State of Maryland engages in an extensive program of moving annually large quantities of seed oysters from a number of specified seed areas to widely scattered growing bars within the State. Private planters in Maryland secure their seed from many sources, often from the waters of other Atlantic Coastal States ranging at times as far away as Connecticut and South Carolina. It is apparent that significant differences in average rates of growth appear among the diverse bars within the State, among groups of seed from different sources, and from year to year on the same bar with the same type of seed. It would be of much practical value to have a measure of such differences in order that bars having rapid growth characteristics might be selected for state plantings, that seed oysters from the different seed areas be used where they are most effective, and that planters be able to select seed from sources best suited for growth on their grounds. Information on the rate of survival of seed oysters also is needed in order to calculate yields from the plantings. It is recognized that practical experience has been a useful guide for such operations in the past but information from this source seldom is made public or is not very specific in nature.

The data presented herewith are fragmentary and as yet insufficient to supply the answers needed. However, enough information is at hand to indicate the average rate at which native Maryland oysters grow and the extent of the differences which are found among certain bars. Growth behavior of oysters brought in from outside the State is roughly indicated as are gross differences in mortality rates among different groups of seed planted at the same location.

A number of investigators in other States have followed the growth of oysters through periodic measurements of one or more of the following: Length, width, thickness, total volume, volume of the shell cavity, and total weight. The relationships between these different measurements have been shown and precise charting of seasonal growth of both individuals and groups of oysters has been published. Loosanoff showed significant differences between Maryland and Connecticut seed in both growth and mortality when reared together in Milford Harbor. Butler currently is comparing the behavior of Maryland seed with local seed of the same age at Pensacola, Florida. A preliminary report on growth of oysters held on trays at Solomons, Maryland was made at the meeting of the National Shellfisheries Association in 1949.

While it would be desirable to gather data on all types of measurement indicative of oyster growth such a practice would not be practical when working hurriedly with large groups of oysters in the field. Weight or volume averages are the simplest to obtain but such determinations have been ruled out because many small oysters in the groups sampled adhere too tightly to shells to be separated without destroying the oyster. Also, older oysters often are heavily encrusted with barnacles, mussels, serpulids, spat, and many other organisms difficult to remove. Increases in length, width, and thickness of oysters all are related so that any one might be used as a reasonable index of the oyster's growth. Since overall length is easiest to measure, provides the least per cent of error, and is the criterion by which market size is legally established, that dimension has been selected as the index of growth for the groups of oysters reported upon.

Lunz has pointed out the difficulties of measuring an oyster precisely and the fallacy of reporting their dimensions down to small decimal fractions of a

millimeter. Among the groups of oysters given here most were measured by
vernier calipers which were read to the nearest millimeter. Averages of the
groups also are expressed to the nearest millimeter. The figures represent total
length of the oyster along the longest axis from the hinge end and include the
entire left valve, not just the length represented by the right valve.

Several types of measurements made during the past ten years are included
in the length data. Spat caught on shells in seed areas and then transplanted
to growing bars, where few if any local spat attach, are quite easy to identify.
Measurements of such spat originally consisted of a minimum of 400 random indi-
viduals. In one instance a special study was made where a number of samples of
the same seed were compared totaling over 2,000 individuals. Although the range
in length of individual oysters from the same year class is fairly great, analysis
of the samples indicated that the standard error amounted to well under 2% of
the mean length when samples of 200 individuals were taken. More recent samples
then have consisted of 200 individuals of the same year class which is believed
to be adequate for the purpose of these comparisons. Other measurements of larger
seed of mixed age at times were made on bars where their identity could be followed.

Many measurements also have been made of oysters planted on trays at **Solomons**
and at a few other locations starting with groups of 200 to 500 individuals.
Cumulative mortality sharply reduced the number of individuals in these groups
so that much smaller numbers were present at later measurements. Length data from
all types of measurements will be discussed.

Of primary interest is the rate at which native Maryland oysters normally
grow. It previously has been reported that several cases of exceptionally rapid
growth have been observed where some spat exceeded three inches in length at the
end of the first growing season or at an age of approximately six months and
that individuals of over six inches in length have been observed at the end of
the second growing season. No measurements of average length were made among
these groups. In certain accumulations of obviously old oysters of unknown
age are found where few individuals have reached the legal size of three inches.

A good indication of growth on typical productive rocks can be found in data
accumulated from bar examinations made each fall. In some cases the identity of
certain year classes is quite distinct and while no measurements are made the
proportion of oysters reaching legal size is recorded. The following lists those
recorded instances where oysters of known age first showed 50% or more of legal
size.

1 bar	50% legal	Second fall approx. age 17 months
1 bar	86% legal	Second fall " " 17 "
11 bars	50-75% legal	Third fall " " 2½ years
4 bars	50-75% legal	Fourth fall " " 3½ years

In all of the above cases oystermen worked on the bars during the season of
the examination.

Many measurements of random samples of planted seed follow their growth for
only about one year because of difficulty in making positive identification of
the group under observation beyond that age. However, in a number of instances
growth was followed almost up to the time when the oysters were disturbed by
harvesting operations. Graph No. 1 shows three groups of the set of 1943, two of
which originated on shell planted directly on the bar where the oysters matured
and one which was transplanted from the St. Mary's River seed area. Growth of

seed remaining on the seed area is compared with that planted on Thomas bar in the upper Patuxent. The majority of all three groups were harvested during the 1945-46 season when average growth was well over three inches, the oysters on Parker Moore being somewhat smaller than the others.
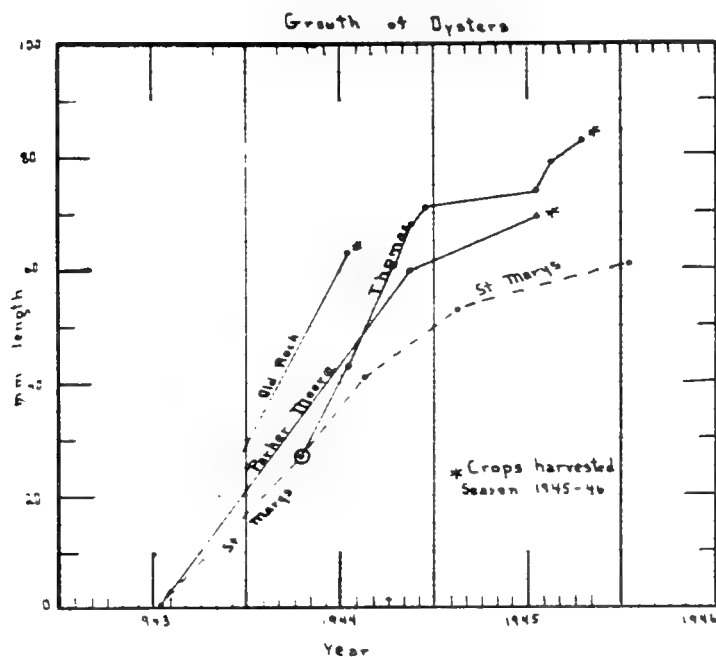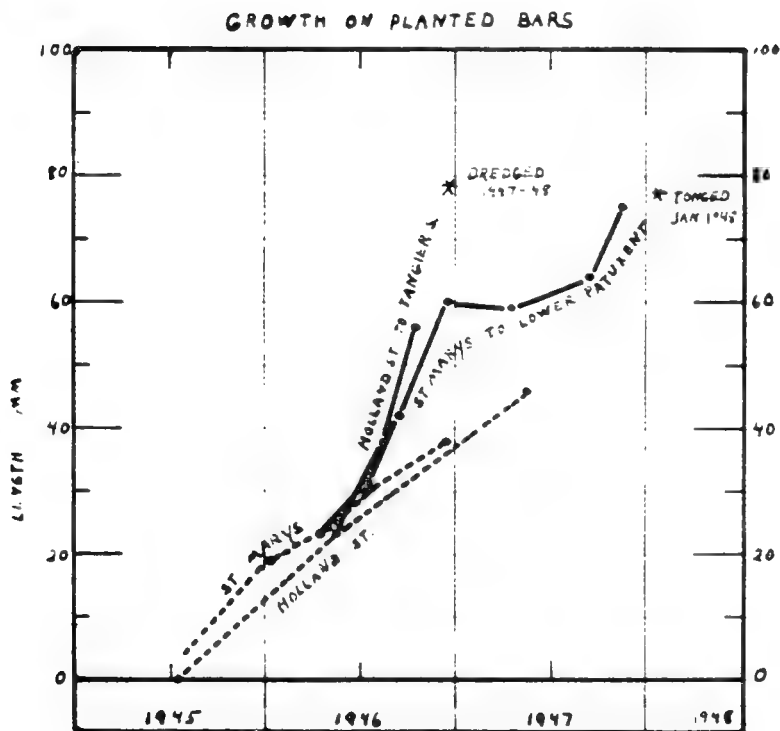

Graph No. 1

Graph No. 2 shows three other groups of seed which originated in seed areas and upon which harvesting operations commenced when the oysters were about $2\frac{1}{2}$ years old. Size at the beginning of tonging in the fall of 1951 was not measured but on the Patuxent it was observed that those in the upper river were noticably larger than those in the lower part. Recent measurements made after the bars had been worked for one season show this difference clearly.

Graph No. 3 shows two groups of seed, one of mixed year class, which were transplanted from seed areas. Most but not all of the St. Marys seed was of the 1945 year class. A slight recession in length which often has been observed during the winter, and the greater extent of fall over spring growth, is illustrated by the St. Mary's transplanting. Here again growth in the seed areas remained much lower than on the growing bars.

GROWTH ON SEED PLANTINGS

Graph No. 2

GROWTH ON PLANTED BARS
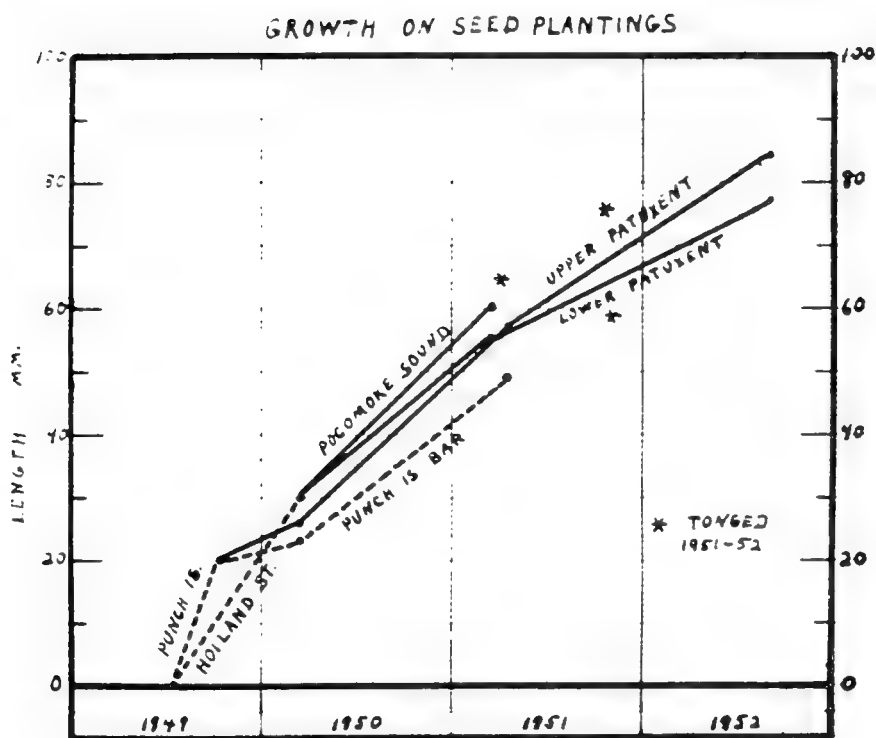
Graph No. 3

Chart No. 4 shows a scatter diagram of all growth measurements where age of the seed was known. Some represent the relatively slow growth in seed areas and others the typical growth on good, growing bars. It is assumed that most of the oysters had set after July 1 of the initial season. By labelling the horizontal scale as years of age, it becomes apparent that the oysters observed typically reached about 3 inches (76 mm.) in length at the end of the third growing season. All data above are from the Chesapeake area of Maryland.
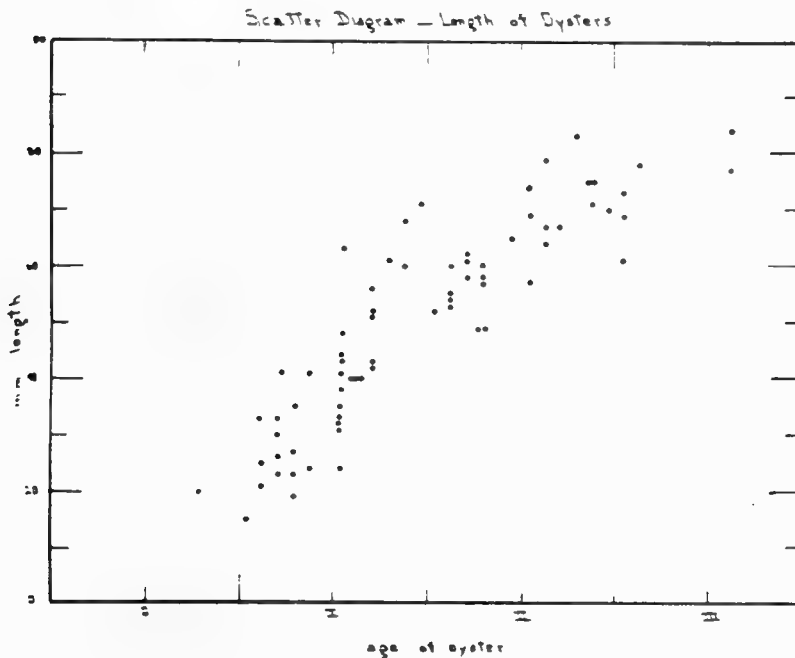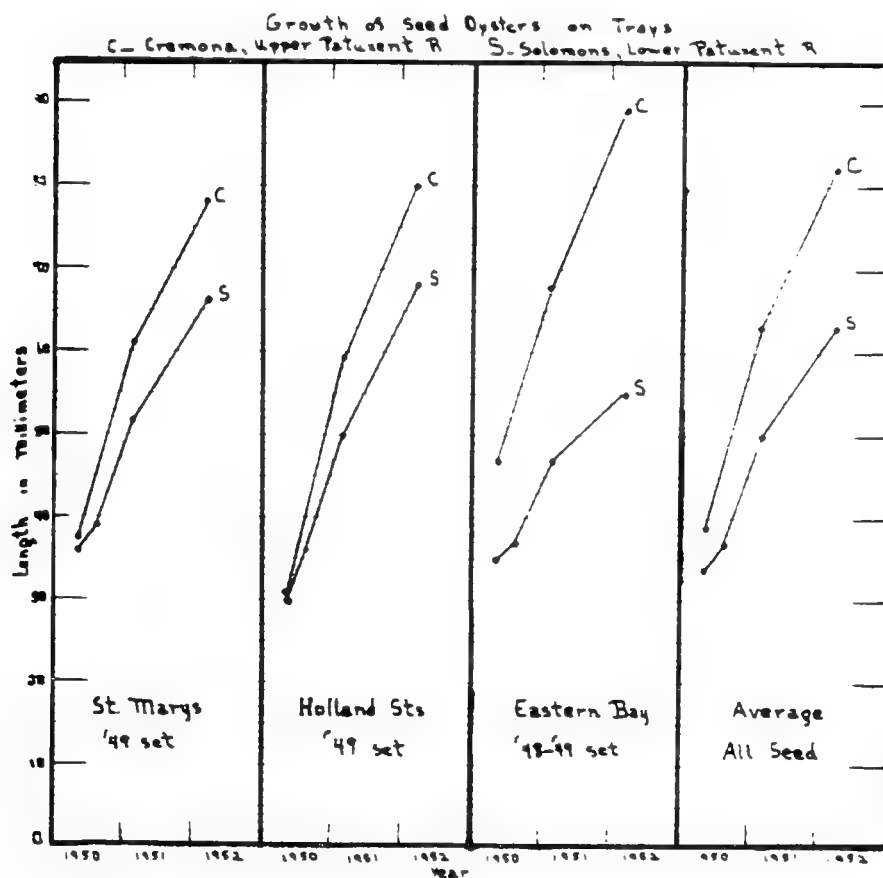

Graph No. 4

Measurements of oysters on trays possess the advantage of following the same group through but are subject to errors caused by mortality and may not truly represent oysters growing on the bottom. To eliminate the latter as much as possible all of the trays included here were resting directly on the bottom, sometimes with short lengths of 2 by 4 boards wired beneath them to prevent too much settling. Difficulty was experienced in recovering trays from deep water with the result that depths of water ranged from about three to five feet over all trays included in these observations.

Most trays originally were placed under the laboratory pier at Solomons on sandy bottom. No great difficulty was experienced here during the first two seasons but subsequent storms shifted the trays and loosened up the bottom so that considerable sanding of portions of the trays has occurred since and rendered

the mortality data for that period unreliable. It also was found that the rate
of growth under this pier was much less than that usually observed elsewhere.
This probably resulted in part from the sluggish current, prevalence of suspended
sand grains in the water, and accumulations of grass and Ulva which often settled
over the trays.

A comparison of the growth of seed from the State's three major seed areas
on the trays at Solomons and on trays at a similar depth in the upper river is
shown by Graph No. 5. The better growth at the up river station is quite marked.
At Solomons none of the groups had averaged market size at the end of the third
growing season while at Cremona the average was well above 76 mm. at the same
age. The Eastern Bay stock at Cremona was partly of older seed than those at
Solomons due to limitations in the amount of this seed then at hand.

Graph No. 5

Graph No. 6 shows the growth of four groups of seed under the pier at Solomons
which exhibited differing rates of growth. All of them grew more rapidly at
first and made more growth during the fall months during the first two seasons.
They show recessions in length during late winter and in mid-summer as they in-
creased in size. Part of this apparent retrogressive growth may have been due
to increased mortality among the larger individuals. Much of it was real, how-
ever, as the oysters had laid down new growing edges well back inside of the

former thin bills which were eroding back and so gave decreased readings of
length. At the same time some increase in thickness occurred. Groups A and
B show significant differences in rate of growth. Best growth of all four was
shown by seed from Holland Straits, a nearby source. This not only grew better
but had the least mortality and attained the largest size. None of the seed,
however, can be said to have done well at this location.

GROWTH ON TRAYS AT SOLOMONS
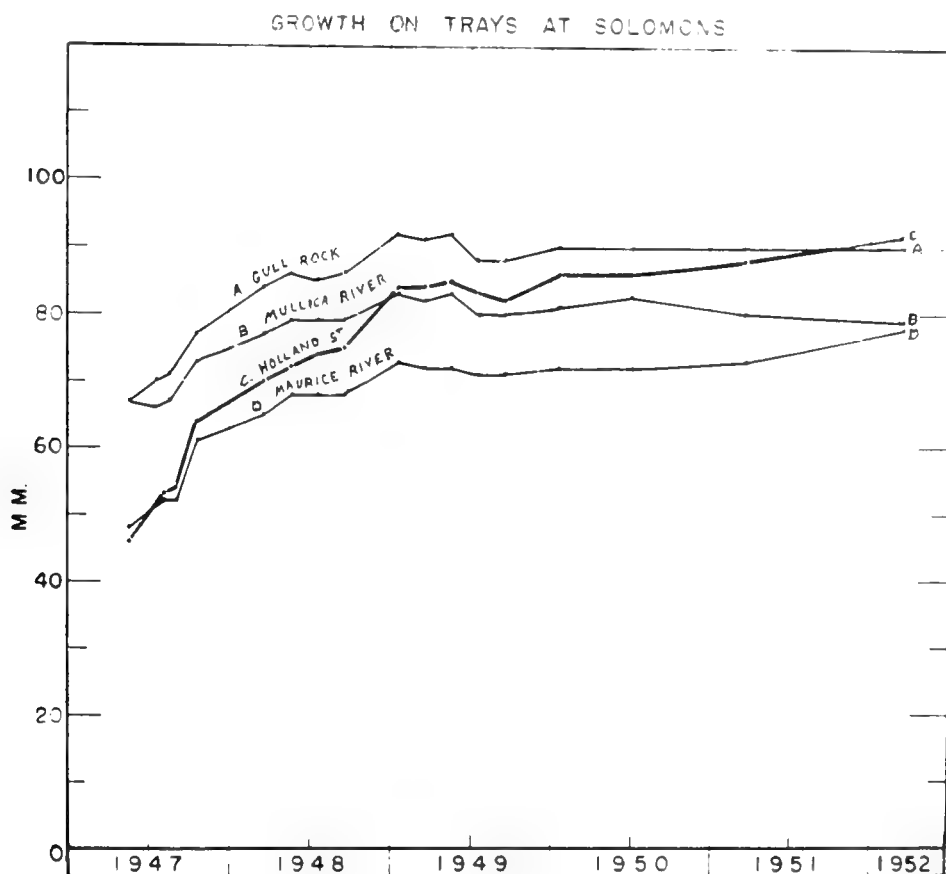
Graph No. 6

The same Holland Straits group which grew best under the laboratory pier is
shown again in Graph No. 7 where it may be compared with similar seed from a
nearby source transplanted to an arm of Chincoteague Bay. Also two groups of
seed from Punch Island Creek bar started at the Chincoteague planting and one
of the same batch started at Solomons are shown. The oysters in the Chincoteague
area grew at a much more rapid rate, had far less mortality and attained a
much larger size than the similar plantings at Solomons. The slow-down in in-
crease of length by the larger oysters during late winter and in mid-summer
again is shown.

Many groups of seed from different sources have been tried at Solomons.
Among some of them mortality was quite high although exact figures could not be
obtained because part of the loss resulted from the smothering of some by sand

and the disappearance of many of the small seed from the trays. These latter probably were carried off by blue crabs. In general, however, it can be said that seed from local sources grew and survived best. Mortalities were highest among seed from areas of higher salinities such as Long Island Sound and the coastal waters of southern areas. These conclusions agree with opinions based on practical experience.

GROWTH OF SIMILAR SEED AT TWO LOCATIONS
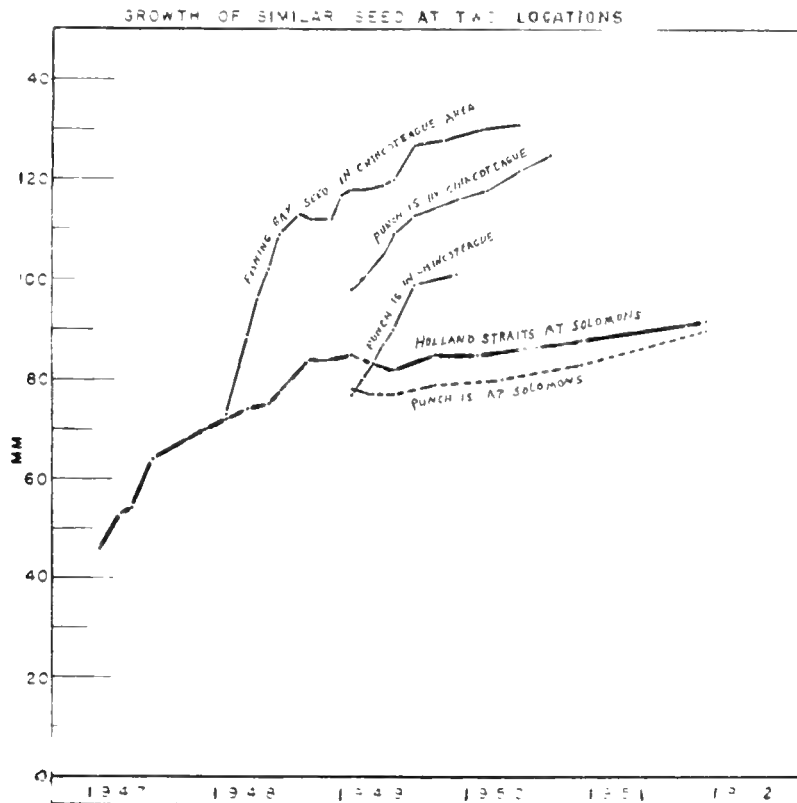
Graph No. 7

As long ago as 1881 Ingersoll, in his publication "The Oyster Industry," wrote: "In general, transplanting young oysters in water similar to that in which they were born, causes them to grow more rapidly; but if they are carried into different temperatures and other strange conditions, they will grow slowly. Thus, in New York bay, the East River and Newark Bay seed far outgrows that brought from Virginia. In the Chesapeake, no doubt, the reverse would be true." These words penned over seventy years ago seem equally true today. That great differences in growth occur among the bars of a given locality also was noted by Ingersoll and it is through recognizing these differences and the differences occurring among seed that the most effective returns can be obtained from a planting program.

In summary of rates of growth under Maryland conditions it appears that in rare cases on certain bars local seed may contain some individuals over three

inches in length at the end of the initial growing season and may average above
legal market size at the end of the second growing season. On most bars mar-
ket size is reached at the end of the third growing season and on a few bars
more than three seasons will be needed to produce marketable oysters. Quick
growing oysters are thin shelled and may break during shucking. In the Chinco-
teague Bay area growth typically is more rapid than in the Chesapeake and seed
oysters from the Chesapeake area do quite well there although comparisons of these
with oysters from other sources are lacking.

SOME OBSERVATIONS ON THE MIGRATIONS AND SETTING OF OYSTER LARVAE

Thurlow C. Nelson
Rutgers University

The two absolute minimum requirements for successful oyster culture are: (1) a dependable supply of seed; and (2) adequate food supplies to assure plump oysters during the market season. Let me dispose of the latter here today with the plea that since it is stored glycogen or animal starch which makes a good oyster and not fat, that henceforth we cease speaking of fat oysters and designate them as "plump".

As we meet here today the New Jersey oyster growers as well as those in some other areas are faced with a critical shortage of seed oysters. Some factors contributing to the situation in this state have been presented by my associates. In the Long Island Sound area the hurricane of November 25, 1950, was responsible for severe losses of seed and young market oysters. There is, however, no cause for alarm since there have been other periods of scarcity of seed oysters, notably after the close of the first World War, when greatly increased industrial pollution resulted in destruction of in-shore spawning beds in the Bridgeport and New Haven areas. The natural high fecundity of the oyster together with the ingenuity, energy and courage of our oyster growers is a combination capable of overcoming any difficulty short of complete ruination of our coastal water through pollution or other causes.

More seed oysters we must have, and as a first step toward this objective let us quickly review our knowledge of oyster larvae to determine what we actually know about this most important little animal, and what we need to know, to the end that we may cooperate with it more intelligently in securing sets of commercial value.

We owe to two outstanding zoologists, Dr. Joseph Stafford of Canada and my father, Dr. Julius Nelson, long Professor at Rutgers College, most of what we know prior to 1920 of the spawning and larval life of the Atlantic Coast oyster, Crassostrea virginica. In 1908 in his report for 1907 Dr. Julius Nelson published a series of 45 drawings of the oyster spat from immediately after attachment where only the shell of the mature larva is shown, to spat ten days old. In the following year he published three excellent photomicrographs showing oyster larvae one day old to approximately six days after fertilization, although he believed the latter to be 20 days of age.

In the same year Stafford published the first of two papers on the larva and spat of the Canadian oyster in which for the first time he showed the foot and eyespot of the larva. Using a plankton net of bolting cloth for the first time in the study of oyster larva, and aided by the very clear waters of Nova Scotia, Stafford was the first to see and to describe the stages of the oyster larva during the latter half of its free swimming existence.

In 1921 I published photomicrographs showing 8 stages from the earliest straight hinge to setting size; and in 1951 Dr. M. R. Carriker presented careful drawings and dimensions of the principal stages of the

larva. Together with the excellent microscopic slides of these and of the
larvae of other bivalve mulluscs produced by Dr. Loosanoff and his associates
in the Milford Laboratory, it should now be possible for any one familiar
with the microscope soon to be able to distinguish oyster larvae.

So much for their recognition. What do we know of their movements,
their distribution, their reactions to currents, tidal and wind driven,
to salinity gradients; of their food and feeding; of their enemies? What
do we know regarding their reactions at time of setting, of factors affecting
early survival of the spat?

1. Distribution: Dr. Carriker has given us in Ecological Mono-
graphs, Volume 21, January, 1951, an excellent summary of observations
published to date while adding considerable data of his own. To this
paper I must refer you for full details since time limitations will permit
me only to touch a few of the points which may be of significance to the
practical oyster grower interested in seed production. In New Jersey
waters observed or recorded spawning of oysters has occurred at or close
to high water. Prytherch reported the same for the Milford, Conn. area in
Long Island Sound. The only exceptions to this in New Jersey have been on
the Cape May flats of Delaware Bay where Mr. J. R. Nelson and Capt. Conahey
of the East Point Oyster Co. have both observed extensive spawning as the
first of the flood tide covered oysters exposed at low water.

Here the early flood tide carries in water which has stood over
the shallow flats several hours. On hot days temperatures of this water
have been recorded as high as 106°F. Also because of great activity of the
algae carbon dioxide has been used up, resulting in high alkalinity. We
know that oysters can be made to spawn by raising the temperature sufficiently
high. There is some evidence that high alkalinity may also be a factor
stimulating spawning.

Fertilization is effected and development of the egg begins within
an hour after spawning and within 5 hours the embryos are actively swimming.
I have found early non-swimming cleavage stages of the oyster in numbers up
to 1250 per quart of water, carried along in early ebb tide; hence it is
probable that the young oysters are swept as far downstream as the first ebb
tide will carry them. The younger stages of the larvae are well distributed
in the water, hence tend to be carried in New Jersey some distance from their
point of origin. Because they pull in the swimming organ or velum when
suddenly disturbed, they tend to be collected along tidal slicks and eddies
or in lanes, as Dr. Carriker has pointed out.

Many an oyster grower has asked me to explain how one ground can
catch a heavy set while an adjoining one shelled at the same time receives
little or no set. I can only point to Dr. Carriker's observations and our
own, and tell you of two wire bags of sea clam shells placed 4 paces apart
on the Cape May flats this summer. One is literally plastered with spat,
the other has practically none.

In 1917 I published the results of the first survey of oyster larval
distribution made anywhere in the world, and showed that the larvae tend to
move upstream from the parent oysters before finally attaching. In the same

year my father published similar results from his studies in Richmond Bay, Prince Edward Island, Canada.

In their vertical distribution in waters with sharp salinity gradients I showed in my annual reports between 1924 and 1931 that the larvae accumulate in greatest numbers just above the level of most rapid salinity rise. In 1930 it was demonstrated in the laboratory that mature oyster larvae ready to set will rise up from the bottom and swim actively in response to even slight increase in salinity. On the contrary, with slight decrease in salinity they draw in the velum and drop to the bottom.

It is well known that heavy sets of oysters often occur on the bottoms of boats or of floats anchored in oyster bearing regions. Such a heavy set on the outside of the bottom of a floating box was pictured in our report published in 1917. In 1920 approximately 100 eyed oyster larvae were obtained in two dips of a plankton net at the surface of a tidal creek entering Little Egg Harbor, N. J. The tide was in early flood stage. It is evident therefore that oyster larvae ready to set do come to the surface at times.

On the contrary over the planted grounds and natural beds of Delaware Bay it is necessary to lower the hose intake to within a foot or two of the bottom in order to find the mature larvae, whereas the younger stages are found at all depths. Since the flood tide runs for approximately an hour longer on the bottom than at the surface in Delaware Bay it is obvious that any object floating at this depth must be carried upstream with each flood tide farther than it could be transported back again by the ebb. From field observations and from laboratory studies we believed by 1921 that the mature larvae were actually upon the bottom for at least a part of the ebb tide. Dr. Carriker from his studies in four different areas on the New Jersey coast extended our studies and in 1951 published his confirmation that mature oyster larvae do actually rest on the bottom during much of the ebb tide.

We have told you of the intensely heavy sets which strike year after year on the Cape May shores of Delaware Bay. Younger stages of the oyster larvae are seldom seen here, but the eyed larvae ready to set appear suddenly at approximately mid-tide and at the surface in such numbers that over 1,000 have been taken in a 5 minute tow with a #8 bolting cloth net. As the speed of the flood tide slackens toward high water the larvae disappear from the upper water and settle to the bottom 6 to 7 feet below where they set. Last year there was on demonstration a sea clam shell bearing as high as 674 living spat per square inch. Shells bearing comparable sets are on exhibit here today. Only on two occasions have we found any larvae here during ebb tide and these were in surface currents away from the shore set up by strong east winds. Oyster growers who have shelled this shore for years have noted the sparse sets when strong offshore winds have continued day and night during the spawning season. Southerly and onshore winds on the contrary they have associated with good sets.

Circulation patterns of the water in Delaware Bay are beginning to emerge as the result of extensive current studies. On the basis of present knowledge we believe that the younger larvae are carried well down the Bay toward Brandywine Shoal but as they get older and spend more time at lower levels they are carried by successive stages back toward the beds from which they originally came.

Coming in toward the Cape May flats with the flood tide and at levels close to the bottom they are carried to the surface as the incoming tide strikes the steep offshore face of the outer bar and is deflected upward. With southerly or onshore breezes they are carried the quarter of a mile from the edge of the bar to the very shore transported by surface wind driven currents. With strong offshore easterly winds, however, they are carried away from the shore in surface currents and may at slack water settle to the bottom to give heavy sets on the shells planted as far off as Deadman's Shoal 5 miles away.

Dr. E. B. Perkins working with us in 1929 to 1931 showed that oyster larvae are found in greatest numbers in their vertical distribution where the current is the strongest. This is true, however, only when the salinity is relatively uniform from top to bottom. With salinity gradients as they occur in Barnegat Bay, however, the greatest numbers of oyster larvae were found just above the level where a sharp increase in salinity began.

The studies of larval distribution conducted in New Jersey since 1916 and confirmed by Roughley of Australia with the larvae of Crassostrea cucullata lead very clearly to two important conclusions: First, larvae of different ages show different circulation patterns; and second, oyster larvae are not uniformly distributed through the water but occur in lanes or in local swarms as it were. As touching the larvae reaching setting size, Dr. Carriker's figures from New Jersy coastal estuaries are significant. Speaking now of mature and eyed larvae he states: ". . . on the ebb there were almost twice as many of these larvae directly on the bottom as off the bottom; and 32 times as many on the bottom on the ebb as on the flood."

How far may oyster larvae be carried from the beds where the eggs were spawned? I know of only one case in which there is absolute proof. Dr. C. Roy Elsey of British Columbia, our first Ph.D. at Rutgers, told me of finding one spat of the Pacific or Japanese oysters Crassostrea gigas, on a boulder of some 5 to 7 tons five miles from the only bed of these oysters within approximately 30 miles. That boulder wasn't dropped off an oyster boat; that spat came from an oyster larva which was carried those five miles by currents.

We do have good evidence that a fine brood of oyster larvae of setting size was carried from the mouth of Cedar Creek, Barnegat Bay some three miles to the vicinity of Barnegat Pier in a single evening spring tide with strong southerly wind. Up to the last afternoon they had been concentrated on the natural bed at the mouth of Cedar Creek.

By placing an 18 inch disk under the strainer on the lower end of the hose used to pump samples, Dr. Carriker was able to draw up water from the very bottom containing mature and eyed larvae. Dr. Prytherch, studying distribution of larvae in Milford Harbor, Conn., believed that oyster larvae of all ages remained on the bottom except during slack water when the l currents are at a minimum. Thus he explained the tendency of spat to settle close to the parents.

We know, however, that heavy spatfalls often occur far from parent oysters as they have done every year but two since 1927 on the Cape May flats

of Delaware Bay. One reason for heavy sets near adult oysters is the
fact that they capture large numbers of their young in the water which
they strain through their gills. These larvae entangled in mucus are
either discarded at the edges of the palps and extruded with the rejecta
of they enter the stomach, are sorted out with the sand grains and sent
down the intestine in from 20 minutes to half an hour. Eyed larvae have
been seen to crawl out of both rejecta and dejecta of the oyster, to extrude
the velum and swim off. Proof that such larvae have set and produced viable
spat was presented to you at an earlier meeting. A practical experiment de-
signed to test the effects of parents on setting is described and illustrated
in our exhibit.

2. Attachment: It is of considerable interest that the setting stage
larvae of the oyster, the sea mussel, the southern hooked mussel, and the
horse mussel all of which attach above the bottom, develop an eye spot where-
as larvae of burrowing forms such as the hard and soft clams and Teredo do
not. Oyster larvae of setting size have been followed both in nature and in
the laboratory and in neither have they set as long as they are in the light.
Remove them to the dark room and the majority will have set in half an hour.
On the Cape May flats larvae continue to crawl about on a shell in counter
clockwise spirals until reaching the edge of the shell where they have dug
their way under to attach on the lower side. This year for the first time
in our experience our test shells have shown a very great preponderance of
setting on the upper as opposed to the lower side. Several of the test shells
of the hard clam Venus are put out and removed every 24 hours, one with con-
cave surface facing up, the other facing downward. Counts as given are for
the inner concave surfaces only. The record to August 4 shows a total of 650
spat on the downward facing shells and 2174 on the up facing shells. On only
one occasion thus far this summer has the count on the downward facing shell
exceeded that on the up facing shell. Since July 15 when setting of importance
began this summer, the water has carried a heavy load of diatoms and dino-
flagellates causing a turbidity disc to disappear at depths between two and
three feet. It is possible therefore that these algae have cut off so much
light that not enough has reached the bottom even in 5 to 6 feet of water to
stimulate the larvae to crawl to the under sides of the shells.

Oyster larvae in setting often accumulate in slight depressions of
furrows in a shell. The shell of the Angel Wing, Pholas costata, has both
furrows and pits on its inner surface. One such shell from our Cape May
flats has a single oyster spat in each pit but none in between. Larvae
which arrived after all the "berths" were occupied apparently moved on to
seek other quarters.

Every oyster planter knows the value of clean shells, but no one
with an extensive planting program can possibly get all his shells over
after spawning has begun. Washing the slime and oyster blood from shells
as they go up the conveyor from the shuckers bench to the shell pile will
remove animal matter which encourages early fouling of the shells when these
are planted the next summer. Washing done at once is far more effective than
the very incomplete cleansing shells receive when driven overboard with streams
from a hose.

Of all the fouling organisms one of the most effective in preventing
set is the group of calcareous bryozoa often referred to by some oyster

planters as "coral". Studies made at the Chesapeake Biological Laboratory at Solomons show how the effectiveness of oyster shells as cultch has been largely destroyed by these bryozoa within three weeks.

3. **Enemies:** Although mature and eyed oyster larvae may pass unharmed through the digestive tracts of adult oysters, the younger stages at least are not so fortunate when engulfed by Ctenophores or comb jellies, Mnemiopsis leidyi. These have no stinging cells like the jelly fishes, but capture their food through their 8 rows of paddle plates. Plankton including oyster larvae, when struck by the plates is carried down into a deep gutter along the base of the row whence it is transported by cilia to the digestive tract. I have found 120 straight hinge larvae in the digestive cavity of one large Mnemiopsis, most of them already but empty shells. At times we have found this comb jelly up to 60 per cubic yard of water. It is probable that all plankton feeders are enemies of oyster larvae to a greater or less degree. Where oyster spawning is spread over a long period in the summer the number of oyster larvae surviving at the end of their two weeks free floating life may be insufficient to leave any survivors after the predators have taken their toll.

4. **Practical considerations:** As we first showed in 1916, and as Dr. Loosanoff has abundantly proven for many years in Long Island Sound, microscopic examination of the water for indications of times of major spawning and setting has proven valuable to the oyster planter in gauging his shell planting. Where larvae are sufficiently abundant to show their presence in swarms surveys of the water may reveal areas to which eyed larvae are brought by the currents but where absence of cultch has made it impossible for the larvae to reveal their presence. Also unless test shells are very carefully and closely followed during the setting period the spat may be destroyed so rapidly by drills or other enemies as to give the impression that no set has occurred.

Obstructions to movement of the tide will produce eddies which often act as traps to capture the larvae as they pass by. This is one of the chief advantages of planting shells in chicken wire bags or in windows.

Finally it must be emphasized with all the force at my command that any oyster set whether light or heavy is valuable only to the extent that the enemies of young oysters are controlled. We are far behind the land farmers and the entomologists who show them how to fight their pests. A good beginning was made during the depression of the early nineteen thirties and in the federal project for Oyster Pest Control but even the lessons learned then have either been forgotten or are largely ignored.

With wages and the cost of boats and equipment where they are today we can ill afford to continue to support the host of star boarders which live on the oysters we hope to raise and sell. As yet we have but few effective means of fighting the oysters' many enemies, but these few should be employed to the full. The biologists who labor to solve your problems are anxious to develop newer and better methods, but only as present means of control are tried in the fires of practical usage can we detect the weakness and so evolve less expensive and more effective ways of keeping these enemies in check. Far more research work by competent scientists is needed to seek out the weak spot in the enemies' armor where it may be most successfully attacked. Research and its widespread application have made our modern agriculture. The can do as much and more for oyster culture.

THE WANDERINGS OF SMALL CLAMS

Osgood R. Smith
U.S. Fish & Wildlife Service
Newburyport, Massachusetts

Most of us are in the habit of thinking of bivalve mollusks as rather sedentary animals after they have developed shells and have settled out of the plankton. In the case of the soft clam, Mya arenaria, small individuals 1.5 mm., or less, in length may be found dug into the mud just like their elders, and, since the mud is their natural habitat, one is likely to assume that the small clams will normally stay there. On the other hand, anyone who has examined New England clam flats knows that you often find clams from two to twelve mm. long attached by their byssuses to floating detritus and algae, or being washed about by wavelets at the water's edge.

Sometimes enormous numbers of these clams are being rafted about at the mercy of waves and currents. A spectacular example of this was seen at Houghs Neck, Quincy, Massachusetts last summer. In that case, green algae floating in two or three feet of water had formed mats a foot or more thick, and these drifting mats were literally packed with small clams. There also were windrows of dead clams on the beach, yet those that were alive and had dug in numbered 12,000 to 16,000 per square foot, based on actual counts from two separate one-quarter-square-foot samples.

The fact that small clams are rafted about and carried by currents was quite fully described by James L. Kellogg in 1901 (Bulletin of the U. S. Fish Commission, Vol. XIX), and many of us have seen it; but never-the-less there seems to have been a general assumption that the habit is not "normal". We have assumed, rather, that the rafted clams have been accidently washed out of the flats and that the byssus is a sort of life line, to enable them to get back in as soon as possible. This idea has persisted in spite of the fact that Kellogg not only describes the byssus and its function, but also tells how a six-millimeter clam in an aquarium dug into the mud and came out again a half dozen times in three days.

We have observed clams 4 to 12 mm. long doing the same thing in shallow pans in the laboratory. The clams would dig into the soil soon after being placed in the pan, and then after 15 or 30 minutes some would come up and move along, using the foot much as a snail does. The shells were supported so that the dorsal or hinge side was up. Where the soil was soft, the wandering clams would plow a small furrow as they moved along with their umbones just exposed. They usually would move slowly but rather steadily for 15 or 30 minutes, making a furrow 4 to 7 inches long, and then tip their posterior ends up and dig down.

The important question, of course, is not whether or not clams can move about, but what proportion moves about and how important this movement may be in determining how many clams will remain on any one flat. If most of them move about, or are moved about until they are nearly a half-inch long, then we must try to attract them at this size and induce them to dig

in if we are trying to increase natural sets. Also, any population estimates based on clams less than half an inch long will be of little or no value in estimating future production. For the same reasons, transplanting or collecting the very small clams would be of little or no value.

The field data being gathered by the Fish & Wildlife Service Clam Investigations at Newburyport, Massachusetts indicate that small clams move about a good deal, that this occurs at all times of the year regardless of storms or calm weather, and that the proportion moving about is large enough to be of great importance in determining the future population of clams on a flat.

To shed a little more light on the stability of young clams, we have collected and counted those that moved into a square foot area during each two-week period throughout most of a year. Trays having screen bottoms were filled with clamless mud, set down flush with the flat and left for two weeks. At the end of this time each tray was taken up and replaced by another. Thus each bi-weekly sample contained only those clams that had moved into the tray during the previous two weeks. Each time these trays were taken up and replaced, a sample was taken of the adjoining flats as a control, to show how many small clams there were where there was no time limit on the period over which they might have accumulated.

We found small clams 2 to 12 millimeters, in our bi-weekly collectors and in the surrounding flats at all times of the year. There was no sign of growth as would be expected if the clams were staying in one place. Furthermore, when they were more abundant in the surrounding flats (the controls) they were also abundant in the bi-weekly collectors. In both series the clams were fairly abundant in May, there were fewer in June and July, and they were most abundant in August and September. For example, on one flat the controls, or samples of natural flat, had 20 to 40 per square foot in June and July, and 400 to 460 in August and September; while the bi-weekly collectors had 11 to 20 for the low and 118 to 150 for the peak in abundance. On the other flat the numbers were not as high, but the numbers in the bi-weekly collectors still followed closely those in the flat. Once there actually were more in the collector than in the surrounding flat.

It should be emphasized that the mud in the bi-weekly collecting trays was flush with the sides and the whole thing was down flush with the surrounding flat. In fact, after they had been in place a day they were hard to find, so they did not offer an unusual barrier or eddy to collect floating clams faster than the surrounding flat.

From the above series of samples it is obvious that both the numbers and the sizes of clams coming into a square foot area in a period of two weeks follow very closely the numbers and sizes in the open flats where they have had all season to collect. I think this is the result of large scale movement and re-sorting of these byssus-bearing clams. The peaks in abundance do not correspond with storms, which pretty well rules out the possibility that the clams were accidently washed out of some place and carried by currents.

Additional evidence of large scale movements of small clams was

secured from the length frequencies of natives that appeared in some plots
of transplanted clams. These plots had been protected from horseshoe crabs
and green crabs by one-inch mesh chicken wire staked down over them. In
1950 we had a crop of small clams that remained under the wire and grew rap-
idly, so that they were two inches long by the fall of 1951. Neither natives
nor transplanted clams survived where they were not protected. In 1951, the
byssus clams were again plentiful, both in the plots set out in 1950 and the
new ones set out in 1951. They were found in monthly samples throughout the
year, but they showed no growth. Their length frequencies remained very
similar to the byssus clams found in the bi-weekly samples. We have no idea
why the clams stayed under the wire one year and did not the next, but the
resuts show that the movements can be a major problem in clam culture.

METHODS FOR THE STUDY OF OYSTER PLANTINGS

Sewell H. Hopkins and R. Winston Menzel
Biology Department, A. & M. College of Texas

Introduction

The methods to be described were designed for a study of experimental oyster plantings on a commercial scale, with the following objectives:

1. To test the relative merits of different locations for commercial oyster growing.

2. To test the relative effects of planting oysters at different seasons of the year, and to find out what time is best for planting.

3. To test the relative merits of seed oysters of different sizes and ages.

4. To determine the effects of different concentrations of oysters, and to find out what density of planting will give the best results.

5. Most important of all, in our opinion, to determine how long planted oysters should be left on the bottom before they are harvested, and what time of year is best for getting the largest and the best yield of oysters. Every farmer knows that a crop must be harvested at exactly the right time in order to get the best yield, as to both quantity and quality. The same thing applies to oyster crops, but oystermen have had to guess whether it is better to harvest now or wait until later. The exact knowledge necessary for judging the best time of harvesting has not been available to oystermen. The main objective of this paper is to describe a procedure for the type of study which will provide this knowledge.

Criteria for Success in Commercial Oyster Planting

Some oystermen and oyster biologists might be inclined to choose one location over another because less mortality occurs at the first location; in other words, the survival is better. Others might be inclined to judge growth, and to prefer a particular location because oysters will grow faster and larger there than elsewhere. We prefer to use the criterion which most oystermen use in practice: the net yield, which is the number of bushels of live oysters which can be harvested, per bushel of live oysters planted. The net yield is the end product of the counter-action of growth and mortality.

A location with high mortality but excellent growth may give better net yields than locations which have low mortality but slow growth. On the other hand, in a good growing area which is badly infested with oyster enemies there may be a low net yield in spite of a very good growth. The possible net yield can be calculated for any given type of seed in any location for each month, from time of planting on, if one has reliable figures on the two controlling factors, growth and mortality.

Methods for Getting Growth Data

It is easy to get reliable figures on growth rate. All that is necessary is to tong up representative samples from each planted plot and to measure them. Measurements may be linear dimensions -- length, width, thickness -- live weight, weight of meat, live volume, inside shell volume, volume of meat, etc. We have obtained sufficient data on all of these types of measurement to construct conversion curves so that one can be converted into another -- for instance, in our region oysters of a certain length will average so many grams weight. However, the one measurement which is absolutely necessary is the number per bushel -- the number of oysters required to fill a bushel measure, or whatever unit of measure is used in the local oyster industry. As oysters grow larger, the number required to fill a bushel naturally gets smaller. This decrease in number per bushel is conveniently shown in a graph of number per bushel each month; after several months the shape of the curve is well enough established so that it can be projected into the future if desired.

Methods for Getting Mortality Data

It is as difficult to get reliable mortality data as it is easy to get growth data. The usual procedure of tonging samples and counting boxes, to get the percentage of boxes in the total number of live oysters and boxes, is of no value whatever if any large time factor is involved. This statement can be proved up to the hilt by anyone who will give it a thorough test. The "box count method" of determining mortality will not give a true picture over a period of months because boxes do not separate at a constant rate under all conditions. When buried deeply in silt, boxes may remain intact indefinitely -- in fact, they may become fossilized in the closed position and dug up as fossil boxes millions of years later. On the other hand, boxes on hard bottom exposed to wave action may separate almost immediately. What is more to the point, hinge ligaments decompose and weaken, so that the valves separate, faster in warm weather than in cold weather. When warm weather brings an increase in mortality rate, the boxes also break up faster so that there may be no increase in percentage of boxes from month to month. When cold weather slows up the mortality, boxes stay together longer so that the percentage of boxes may show more increase during periods of low mortality than during high mortality periods.

A considerably more reliable picture of mortality may be obtained by counting all attached left valves, as well as boxes, as dead oysters. But even this does not show the true picture, because attached left valves become separated from the clusters in course of time, and because single oysters which have died will not be represented by attached shells.

In tray experiments it is easy to get exact figures on mortality because loose valves as well as boxes are recovered. The only way you can get figures anywhere near as reliable on mortality in bottom plantings is to measure off a number of representative sample plots and then tong everything off of each plot, shells and all. Theoretically, the percentage of boxes and left valves (loose and attached) in the total number of live oysters, boxes, and left valves, should give you the percentage dead. However, we have found by experiment that it is impossible to recover 100% of the oysters and shells by tonging, and shells are not recovered in as high a percentage as live oysters. Two methods are available for getting better figures on mortality. The one involves the supplementary use of tray experiments, and the other can be used when it is impractical to conduct tray experiments along with the bottom plantings.

Use of data from tray experiments to supplement data from samples of plantings:

Wherever practical, a number of trays, securely covered to prevent loss of oysters, should be placed on the planted plots and filled with oysters of the same stock used on the bottom. These trays should be started at the time oysters are planted and should be examined not less than once every month. By this means one can obtain exact data on the mortality rate during each month, from time of planting to time of harvesting the bottom planting. From these data a curve of cumulative mortality can be calculated. In order to draw this curve in such a way that it can be compared directly with the data from tonged samples, it must start with the percentage of dead present in the seed stock when planted. It is impossible to plant a large area with seed oysters which are 100% alive, as the amount of handling and exposure which would be involved in culling several hundred bushels of oysters down to live oysters, only, would itself cause the death of many oysters. Therefore planted seed always contain a significant percentage of boxes and shells; the exact percentage is determined from samples taken from the boats at time of planting. As a result, the percentage dead in subsequent months is not the percentage of mortality which occurred after planting, but the percentage dead of the total number, live and dead, which was planted. However, if the number of bushels planted and the number of live and dead oysters per bushel is known, it is easy to calculate the number of live oysters planted, the number dying each month, and the cumulative percentage mortality each month from time of planting to time of harvesting. This procedure is based on the assumption that mortality in the covered trays is the same as on the bottom.

Estimation of cumulative mortality without the use of tray experiments:

If it is not practical to use tray experiments, or if it is suspected that tray oysters do not have the same mortality as those planted on the bottom, the cumulative mortality can be estimated by the following procedure:

On a graph of percentage dead each month, plot (1) the percentage dead when planted (including left valves as well as boxes) and (2) the percentage dead at the time of the final quantitative sampling of measured sample plots, calculated as follows: Subtract the number of live oysters on the planted plot, as calculated from the final quantitative samples, from the number of oysters, boxes, and left valves planted. The difference is the number dead. This number, times 100, divided by the number of live and dead (boxes and left valves) planted, equals percentage dead at time of final sampling.

If mortality rates were constant at all seasons, a straight line from the percentage dead at time of planting to the final percentage dead would be the trend of the cumulative percentage dead. But mortality rate is not constant at all seasons; it is higher in warm seasons and lower in cool seasons. Therefore, instead of a straight line, we must draw a line which slants upward more steeply during warm months and less steeply (tending to level off) during cool months. We now have a curve representing cumulative percentage dead, by months, on the basis of the maximum possible estimate of mortality. This is the maximum possible estimate because it assumes that there were no more survivors than the number of live oysters actually tonged up in the final quantitative samples, or in other words, that recovery was 100%. However, it is known to be practically impossible to recover 100% of the oysters on the bottom. Experiments indicate that 80% is a good recovery by tonging, even of live oysters, and that shells cannot be recovered even with that much success. We take 70% recovery to be about the minimum which can be expected, where live oysters are concerned. If the live oysters recovered

are only 70% of the number actually present, then the true number of live oys-
ters is 143% of the number indicated by the final samples. The true number of
live oysters thus calculated times 100, divided by the number of live oysters
and dead oysters (boxes and left valves) planted, gives the final percentage dead
on the basis of the minimum estimate of mortality. A curve is fitted from the
percentage dead when planted to this figure for the minimum estimate of final
percentage dead, in the same way as for the maximum estimate. The cumulative per-
centage dead each month must fall between the points for that month on these two
curves.

After the curves for maximum and minimum estimated percentage dead are es-
tablished, maximum and minimum curves for cumulative mortality can be calculated
in the same way as when tray experiments are used as a source for data on mor-
tality (see above).

Regardless of whether supplementary tray experiments are used or not, the
final step is to calculate the number of live oysters still present each month
on the basis of the estimated percentages of mortality.

Calculation of Net Yield

We started with a knowledge of the number of bushels of seed planted, the
number of oysters per bushel, and the percentage of dead in the total number of
oysters (live and dead) planted, so that we know (a) the number of live oysters
planted, (b) the number of live oysters required to fill a bushel, and (c) the
number of bushels of live oysters. From monthly samples, we now know the number
of oysters required to fill a bushel each month, from time of planting to the end
of the experiment or until time of harvesting. Following the procedures outlined
above, we have estimated the number of live oysters present each month. The next
step is to calculate the net yield possible in each month. This is done in two
steps: (1) Divide the number of live oysters present each month by the number re-
quired to fill a bushel; this gives the number of bushels of live oysters pre-
sent. (2) Divide the number of bushels of live oysters present each month by the
number of bushels of live oysters planted. This gives the net yield, the num-
ber of bushels of live oysters present per bushel of live oysters planted.

Application of Net Yield Calculations

We have now shown that the net yield can be calculated for each month after
planting. When net yields are plotted on a graph, by months, they always show
one of these two trends: either (a) the net yield decreases from the time of
planting, so that it is never possible to harvest as many bushels as were planted,
or (b) the net yield increases month by month to a peak a certain number of
months after planting, and then declines. In the latter case, there may be later
peaks also, but they are never as high as the first one. Therefore, whenever
economic factors permit, planted oysters should always be harvested at the time
of the first peak. The yield possible at that time may be twice or even several
times as great as it will be the next season. This is not a condition peculiar
to Gulf waters; the same thing is shown by analyses of data collected by the
Chesapeake Biological Laboratory, and we think that it applies everywhere. In
some places the largest possible net yield -- that is, the peak in the net yield
curve -- may come only 2 or 3 months after planting; in other places, where
growth is slower, it may come the next season or even later, but in any case,
once the peak is passed the oyster planter is losing money every month he waits
before harvesting his crop.

Such net yield estimates can be used to determine the best possible time of harvesting, which we stated to be the most important of our 5 objectives. Theoretical net yield curves for 6 different sizes of seed oysters illustrate the advantages of small seed over large seed; in fact, the larger sizes never give a 1 for 1 yield and can only yield a loss to the planter. Other applications of net yield curves will probably occur to some of you.

Operations and Problems of an Oyster Census on
Swan Point Bar, Upper Chesapeake Bay.

John R. Webster
U. S. Fish and Wildlife Service
Annapolis, Maryland

The oyster census surveys reported here are being conducted on Swan Point Bar in upper Chesapeake Bay. The Bar is off Rock Hall on the Eastern Shore, approximately fifteen miles southeast from Baltimore, and twelve miles northeast from Annapolis.

Our surveys were designed with a dual purpose. We wished to find a method for estimating oyster populations on bars similar to that off Swan Point. In addition, we wished to apply our findings to Swan Point Bar as an experimental case.

All operations to date have been made from the fifty foot vessel ALOSA maintained by the Fish and Wildlife Service at Annapolis. Our principal gear is a small oyster dredge also known as a hand-scrape. It is proportionately similar to a commercial dredge but measures only twenty-eight inches across its mouth. Its capacity is somewhat more than a Maryland oyster bushel of twenty-eight hundred cubic inches.

Commencement of survey operations in the spring of nineteen forty-nine brought up our first problem, finding the general distribution of oysters within the thirty-three hundred acres of the Bar. Once self-sustaining and yielding well, the Bar had lost much of its productivity during conditions associated with prolonged low salinities in the winters of nineteen forty-five and nineteen forty-six. To remedy this depletion, the State of Maryland started seeding part of the Bar in nineteen forty-seven and has repeated its plantings yearly except in nineteen-fifty.

Our preliminary dredgings were made haphazardly across the bar. They revealed numerous guide situations. The northern third of the bar was muddy with stony ridges. Oyster abundance was spotty. The southern third of the bar was hard bottom with oysters apparently scarce. The central third, wherein the State made its plantings, was mostly hard. In it we found oysters from a depth of twenty-five feet at the outer edge of the Bar to a depth of about eight feet inside, the safe limit for our vessel's draft. Oysters were most numerous around planted spots. Of interest to us from a sampling viewpoint, however, was this: towing the dredge at about two knots, it would fill to overflowing after two minutes in places of greatest oyster density.

We found another situation during preliminary surveys. Dredgings were made on courses. From time to time the dredge hung up on bottom obstructions, with such results as a torn bag or a bent dredge frame. Our analysis of these troubles showed them associated with courses that were not run easterly or westerly. After reviewing all evidences we concluded that our hang-ups were not made on rocks or wrecks, but upon the stumps of old gill net stakes. On Swan Point Bar, stake rows run roughly in an East-West orientation.

The surveyed boundaries of Swan Point Bar and twelve transects across it are shown in Figure 1.
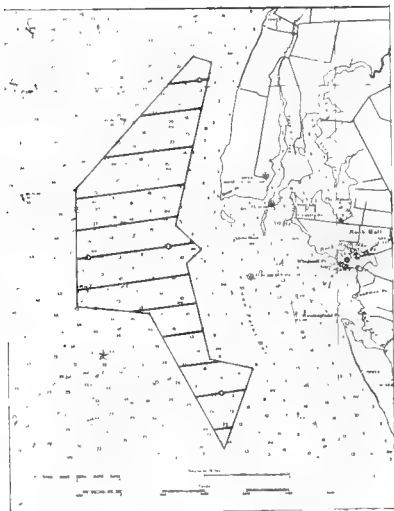
Figure 1.

Experiences of operations during the spring of nineteen-forty-nine became our guides to setting up the experiment in nineteen-fifty.

Our first problem was one of overall sampling procedure: should we make our hauls at randomized spots of run transect lines? We decided upon transect lines for a number of reasons that suited our operationsl opportunities and limitations.

Our second problem was locating these transect lines. We planned to avoid stake lines and to run magnetic East or West since these were easy compass courses to follow. We set up a dozen transects with as many as possible aligned with fixed navigation beacons or prominent marks on shore.

Where such marks were lacking we planned our lines on the chart arbitra-
rily. We did make an effort to space our lines equidistant or nearly so.

Our third problem was to set a standard time of hauling the dredge.
Having in mind the experience of preliminary surveys we set one minute and
a half as standard. This benefited us twice. In the first place, we could
reasonably be sure of bringing on deck a dredge that was not quite full and
therefore having lost none of its catch. In the second place, by using a
short hauling interval we could make more samples during a run. We set up
a practice that has been followed in all our operations: run a steady course,
dredge for a minute and a half, wait three minutes, dredge another minute
and a half, wait three minutes and so on to the end of the run.

Our fourth problem was spotting locations of dredge hauls. This was a
simple matter and we took full advantage of its simplicity. By triangulation,
we determined the ends of all transect lines before running them and buoyed
these positions. On longer runs we set buoys at intermediate positions along
courses so that one buoy could be seen from the next. We marked the positions
of our end buoys on a chart and stepped off distances. Now, by keeping re-
cords of time, and by running our dredge-wait-dredge-wait operations with an
interval timer, we supplied ourselves essentials to plotting: namely, time
and distance with fairly constant vessel speed. Thus, having commenced a
transect run with a haul and its waiting period, and having finished the
run with, let us say, a tenth haul and its waiting period, plotting of haul
locations on the chart posed no problem at all. To be sure we encountered
a few departures from complete regularity but found no difficulty making
allowances for them. I might say here we repeated each run at least once.

Perhaps you can better visualize this description with a picture.

XII
XI
X
IX
VIII
VII
VI
V
IV
III
II
I

LOCATION
RUNS AND HAULS
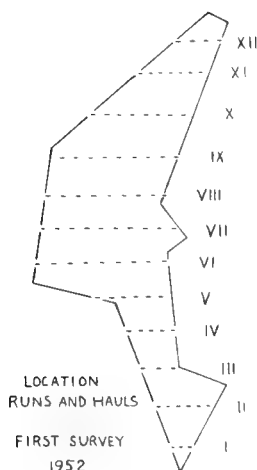
FIRST SURVEY
1952

Figure 2.

Here is a chart that shows our twelve transect runs across the Bar.
Positions and lengths of broken lines represent, as nearly as our plotting
can make it, positions of dredge hauls on the bottom.

This is our first survey coverage of the Bar in April nineteen fifty-
two. Count seventy-nine hauls.

I have taken you now through the operation of bringing bottom samples
to vessel deck. This is by far the easiest phase. Subsequent treatment
includes first the bulk volume measurement of the catch of each haul, in-
cluding oysters, shells, stones and all other natural bottom material except
mud. Next we count all oysters and record their lengths. We volume the
broken shells, count blank shells, count and estimate weights of stones.

This may not sound like much in its telling. I can assure you, however,
that these few basic records supply us a wealth of material. They are
enough to provide data for serious analysis that will tax our capabilities.
Surely we now have information that must be treated statistically if we are
to derive most meaningful interpretation of our work.

At this time and with this time, I can give some examples of the types
of information being found in our data. Perhaps the logical first presen-
tation is our analysis of what the dredge catches. By this I mean all the
natural bottom material. We reduce catch data to volume units of forty
parts per bushel and convert this to amounts per one hundred square yards.
Conversion is made from hauls that covered between fifty and eighty square
yards per haul.
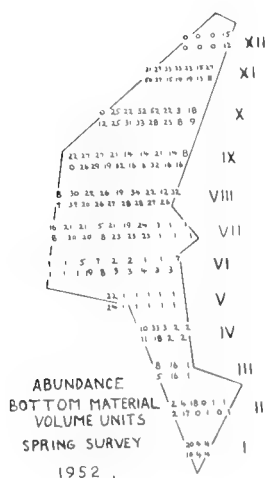
Figure 3.

On the chart, Figure 3, paired horizontal lines of numbers represent middle of a haul location. Each number represents the volume units of bottom material per one hundred square yards as calculated from the catch of that haul. The upper line of numbers plots the first series of hauls made that run. The lower line of numbers plots the second series of hauls on the same run.

I should emphasize here that these numbers represent abundance of bottom material only. What is more, they are measured only of what the dredge caught and retained. We feel sure the dredge did not take everything in its path. This slippage factor, by the way, is another of our problems.

Now, since this is an oyster bar we are primarily interested in oysters. I told you we counted all the oysters dredged up. By simple calculations we convert oyster counts per haul to numbers per one hundred square yards in the region of that haul.

ABUNDANCE
OYSTERS ALL SIZES

SPRING SURVEY

1952
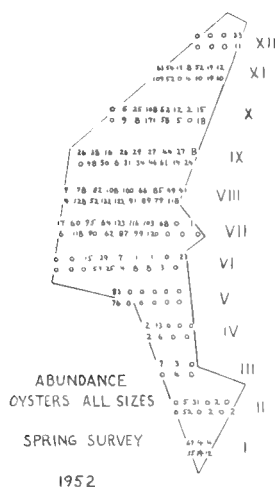
Figure 4.

Here again in Figure 4 are lines of numbers plotted along transect runs. This time, numbers represent oysters instead of bottom material. Data are from our spring survey of this year. Each number signifies total oysters, all sizes, per one hundred square yards. Each number is plotted at a dredge haul location. We tried to make the same number of hauls each time on the run but did not always succeed.

I believe even a casual inspection of this figure suggests another problem that faces us. Sampling error is such that calculations of oyster abundance along a transect line do not agree between repeat runs. We should be hopeful indeed to expect perfect agreement in all cases. Duplication of sampling runs does show the amount of variation encountered by our method. Nevertheless, we may use this information in establishing, at least tentatively, some limits to our range of estimation.

Perhaps I may be forgiven for pointing out, in this figure, a feature that is roughly apparent without statistical treatment. Where dredge hauls of one run indicate oysters scarce, the repeat series of hauls on the same run confirmed this scarcity. Where dredge hauls of another run indicated oysters relatively more abundant, repeat hauls there seconded these findings. We say the dredge did not sweep clean, had a slippage error. It apparently was consistent in this error. Areas of different relative abundance are fairly well defined. Areas of greatest abundance in runs seven and eight coincide with areas of State oyster planting.

As in many other sampling experiments wherein repeats are made, we sometimes find good agreement, sometimes poor. You will recall I mentioned measuring oysters brought up in our dredge. These were length measurements. While we recorded lengths haul by haul I have consolidated them into totals per transect run for illustration.

Figure 5

Here, Figure 5, is an example of good agreement. Data come from
transect run seven this year. We made two runs with the same dredge. I
tallied oyster lengths in five millimeter classes. There are roughly five
of these classes to the inch. Notice some resemblance between the tally
diagrams, particularly in spread. Now see the statistics of these two
distributions. N, or the total sample, is the same for both just the way
we found it without any adjustment. Then, on the left hand, notice the
mean length of eighty-eight point three millimeters with a standard devia-
tion of twenty point eight millimeters. On the right hand, notice the mean
length of ninety-one point three millimeters with a standard deviation of
nineteen point one millimeters. These simple statistics are a mathematical
way of measuring the good similarity between the two samples.

Contrasted with such good agreement, Figure 6 shows a case of poor
agreement. Data are from this year, run three on the narrow southern
section of the Bar. Two series of but three hauls each furnish available
figures. These tally diagrams alone indicate tremendous differences. On
the left hand, N is seven, mean length about ninety-six millimeters. On
the right hand, N is three, mean length about seventy millimeters. No real
need for statistics here though to demonstrate these two samples are differ-
ent. Yet we must use this information in making up meaningful population
estimates at run three, both as to abundance and size range of oysters there.

Figure 6

I could go on and tell you more of our operations and calculations. I could discuss more problems, not to complain but to explain. However, time does not permit.

During this report you have heard about we and I. This is not a one man team. The experiment was conceived by J. B. Engle. Credit for conducting those disheartening preliminary dredgings belongs to Whaley and Davis, men no longer with us. My own participation would have been impossible without able assistance from Paul Heister, our boat captain. Believe me, this last is not just a courtesy credit, as many of you know from your own experience with good boat captains.

Operations and problems. They are ever with us. If any of you, from your own experience, can suggest ways to make our operations smoother and our problems less formidable, I shall say in real gratitude, thank you, Mr. Chairman, Members and Guests.

A WATER QUALITY SURVEY OF HAMPTON ROADS SHELLFISH AREAS

Russell S. Smith
U. S. Public Health Service
Cincinnati, Ohio

The shallow water areas in and near Hampton Roads have always been one of the most important oyster growing sections of the country. The Roads are also one of the finest natural harbors on the Atlantic Coast. With the development of shipping in the area, the port cities of Norfolk, Portsmouth and Newport News grew and developed and, in accordance with customs of the times, discharged their sewage into the nearest body of water. The menace to public health of oysters and other shellfish taken from polluted water became evident to health officials, and the sanitary quality of these water became a matter of increasing concern to the local, state and federal health agencies.

The waters of the Hampton Roads area present an excellent example of the effect of sewage pollution on shellfish growing waters and the improvement possible by means of sewage treatment. Here was a valuable natural resource of extensive, highly productive shellfish beds that were seriously menaced with pollution by the raw sewage from a constantly increasing urban population. As the pollution increased, the use of this great natural resource had to be subjected to ever-increasing restrictions. Finally, with collection and treatment of sewage, the quality of the waters has so improved that the restrictions imposed could be removed over much of the area.

POLLUTIONAL HISTORY OF THE HAMPTON ROADS AREA

Many surveys of the pollution of the waters of the Hampton Roads area have been made. As a result of these surveys, ever-increasing areas of the shellfish beds have been declared unfit for the unrestricted taking of shellfish. Repeatedly, sewage treatment was recommended and warnings were given that, if treatment were not installed, further restrictions would be necessary.

In 1914 the Public Health Service made an extensive survey of these waters. Figure 1. The report of this study condemned the beds in Hampton and Mill Creeks on the North Shore and in the Elizabeth River on the South Shore.

In 1926, as the result of a survey on Hampton Flats and Newport News Bar, the entire north shore of the Roads and all tributaties from Old Point Comfort to a point northwest of Hilton Village were closed to the taking of shellfish for direct marketing.

In 1934 another extensive bacteriological survey of the Hampton Roads waters was made by the Public Health Service in cooperation with the State Board of Health. No additional restrictions were placed on the shellfish growing areas as a result of this survey, but very detailed recommendations were made for the collection and treatment of sewage from the various communities tributary to the waters of the Roads.

In accordance with the recommendations of the 1934 report, the Hampton

SURVEY OF HAMPTON ROADS SHELLFISH AREAS
1949—1950

HAMPTON ROADS SECTION

Roads Sanitation District Commission was created in 1938 for the purpose of collecting and treating sewage entering the waters of the Hampton Roads area. The City of Portsmouth withdrew from the Sanitation District to construct its own treatment facilities. The war caused postponement of the plant construction, although the population of the area increased about fifty per cent.

Increased pollution of the waters during the war led to further restrictions on the use of the shellfish areas. In 1944 the east portion of Craney Island Flats from Hoffler Creek to the Norfolk Harbor Channel was closed to the taking of oysters for direct marketing. In 1946 similar action was taken for a large portion of the Willoughby Bank beds immediately outside the entrance to the Roads.

Thus, during the 1946-1947 oyster season, the waters of the Hampton Roads area were receiving bacterial pollution from raw sewage in sufficient amount that restrictions for oyster taking had been placed upon practically all the growing areas. The only areas from which shellfish could be taken for direct marketing were on the south shore west of Hoffler Creek and the west side of the James River.

After the war the Sanitation District and the City of Portsmouth proceeded vigorously with the construction of intercepting sewers and treatment plants, essentially as recommended in the 1934 survey report. The last plant, that of the City of Portsmouth, went into service in the spring of 1949. There are four plants, two in Norfolk, one in Portsmouth, and one in Newport News. At all plants the treatment consists of sedimentation and chlorination with digestion of the settled solids.

WATER QUALITY SURVEY OF 1949-1950

1. General - This survey of the bacterial quality of the tidal waters in the Hampton Roads area was made during the period from October, 1949, to February, 1950, as a co-operative project of the Virginia State Department of Health and the Environmental Health Center of the Public Health Service. Valuable assistance was furnished by the Hampton Roads Sanitation District Commission, the City of Portsmouth, and the Virginia Commission of Fisheries. The survey places particular emphasis on the waters overlying the shellfish growing areas.

At the time of this survey, certain sewered areas had not been connected to the treatment plants. On the north shore these included portions of Elizabeth City County that discharged raw sewage to Hampton Creek, a samll area tributary to Indian River, the Huntington-Ferguson Park area at the east end of the James River bridge, and scattered homes discharging to Mill Creek. In the Norfolk area there were about 35 public sewers with an estimated tributary population of 10,000 which discharged raw sewage into the Lafayette and Elizabeth Rivers. In the Portsmouth area the Naval Shipyard, the Naval Hospital, the St. Julian's Ammunition Depot, and an industrial area south of the Navy Yard had not been connected to the city sewer system. It is understood that since completion of this survey, most of these areas have been connected to the intercepting sewers leading to the treatment plants.

The shallow waters of Hampton Roads and the vicinity were subdivided

FIGURE 1
SURVEY OF HAMPTON ROADS SHELLFISH AREAS
1949 — 1950
SAMPLE POINT LOCATION
HAMPTON ROADS SECTION

YARDS

NEWPORT NEWS

NEWPORT NEWS
MIDDLE GROUND

THIMBLE SHOAL

PHOEBUS

MILL CR

FORT MONROE

OLD POINT COMFORT

FORT WOOL

WILLOUGHBY BANK

WILLOUGHBY SPIT

SEWELL PT

NAVAL OPERATING BASE

TRAFFIC CONTROL AERO

BB-29

EE-29

LAFAYETTE RIVER

ELIZABETH RIVER

CRANEY I

NORFOLK

LAMBERT PT

K-30   K-21
L-26   L-22
M-29   M-30   M 28   N 26   N-22
N-33   N-31   N-29   N-25
O-37   O-36   O-34   O-32   O-29   O-27   O-19
P-35   P-33   O-30   P-28   P-25
Q-37   P-34   Q-32   Q-30
R-38   Q 36   R-34   R-32   R-24   R-22   R-19   R-17   R-15   R-13
R-37   R-20   R-18   R-16   R-14
S 40   S 38   S 35   S-32   S-21   S-19   S-17   5-16
S-49   T-36   T-24   S-20   S-18   5-18
T-40   T-38   T-35   T-21   T-19   T-18   T-17   T-16
U 39   U 37   V-35   U-29   U-24   T-20   U-19   U-18   U-16
V-41   U-29   U-19   V-17   V-16
W-47   W-40   W-38   W-36   V-24   W-15
X 42   W-39   X-28   Y-14
X 43   X-29
Y-42   V-40   X x-36

DD-45   DD-43
EE 41   FF-37   FF-35
EE-39   FF 32
GG-45   GG-43   HH 41   HH-39   HH-37   HH 34   HH 32
HH-46   43   II-41   II 39   37   II-35   JJ 33   JJ 31   JJ-29
JJ 44   KK-42   KK 39   KK-37   LL-35   LL-33   LL 31   LL-30   KK-25   KK 24   KK-23   KK-22
KA 41   LL-26   NN-21   MM 21   OO 20
NN-27   OO 19
NN 29
QQ-29

FIGURE 2
SURVEY OF HAMPTON ROADS SHELLFISH AREAS
1949–1950
SAMPLE POINT LOCATION
JAMES RIVER SECTION

into 9 areas for sampling purposes. Figures 1 and 2. In these 9 areas, 169 sample points were located to provide representative coverage of each area. Over the period of the survey, a total of 2,656 routine water samples were taken at these points. The least number of samples collected at any point was 5, the greatest number was 40, and averaged 16 samples per point.

Coliform most probable number determinations were made on each sample, planting 3 tubes in each of 3 or more decimal dilutions. All tubes showing gas in any amount within 48 hours were confirmed by inoculating into brilliant green bile lactose broth.

2. Summary of Bacteriological Results - The results obtained from these samples were summarized by tidal cycles, and the median values for flood tide, ebb tide and all tides were determined for each sample point.
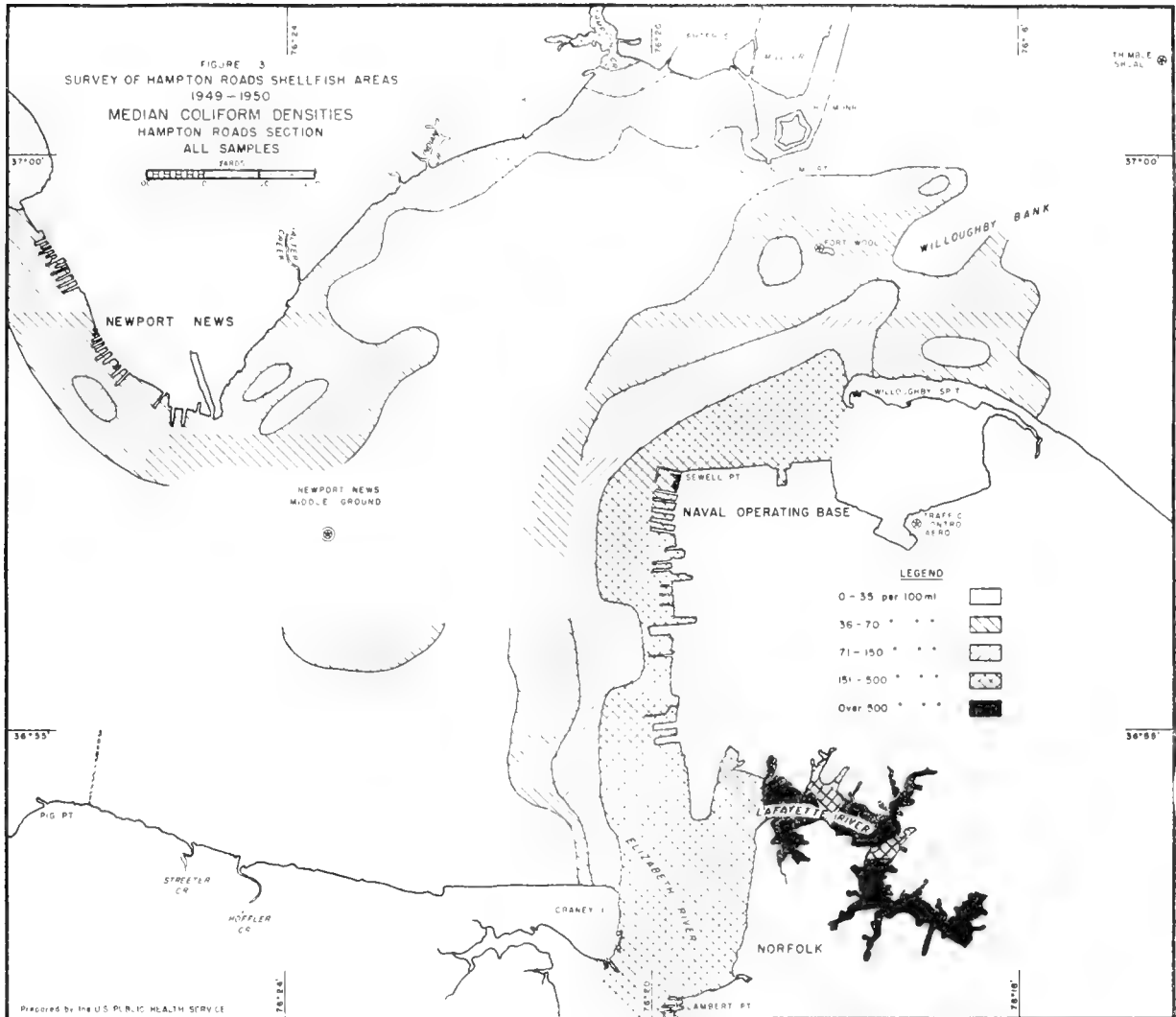
Figure 3 shows the coliform densities, based on median values at the various sample points, found in the waters of the Hampton Roads section. It will be noticed that the water in practically all of the shellfish-producing areas was found to have a quality within the limits prescribed for the taking of market shellfish (median coliform MPN of 70 per 100 ml.). The only exceptions were two samll areas of Hampton Flats, one close to the mouth of Hampton Creek and one just northeast of Newport News Point; and the area along the eastern edge of Craney Island Flats adjacent to the deep water channel. Flood tide results presented an even better picture, particularly on Hampton Flats and Willoughby Bank. The ebb tide samples showed that residual pollution from the Boat Harbor sewage treatment plant at Newport News Point was carried over the southwestern portion of Hampton Flats and that a median MPN in excess of 70 was found as far east as a line perpendicular to shore at Salters Creek. The ebb tide carried the pollution from the Elizabeth River and Norfolk Harbor Channel around Sewell Point and out to Chesapeake Bay. Most of this pollution was carried out through the ship cahnnel north of Fort Wool, but a portion was pushed out over the western portion of Willoughby Bank, particularly adjacent to the north shore of Willoughby Spit.

WATER QUALITY IMPROVED BY SEWAGE TREATMENT

A comparison of the bacteriological quality of the waters of the Hampton Roads area in 1934 and in 1949-50 provides an excellent demonstration of the effects of sewage treatment.

Fortunately, the information given in the 1934 report located each sample point and gave the arithmetric average MPN for each. These sample points were spotted on tracings of the Coast and Geodetic chart (scale 1:20,000) for the area, the average MPN was marked at each point and coliform density curves were drawn. Similar coliform density curves were drawn based on the arithmetric average MPN determined for each sample point in 1949-50. Please note that these curves are based on arithmetric averages of the MPN and not on the medians. The 1949-50 results showed that in this area the averages were about 3-1/4 times the medians.

Figure 11 shows these average coliform densities for the Hampton Roads section in 1934. Note how small a proportion of the water area had an average density less than 100 coliforms per 100 ml. Most of the Roads had a density

FIGURE 3
SURVEY OF HAMPTON ROADS SHELLFISH AREAS
1949 - 1950
MEDIAN COLIFORM DENSITIES
HAMPTON ROADS SECTION
ALL SAMPLES

NEWPORT NEWS

NEWPORT NEWS
MIDDLE GROUND

WILLOUGHBY BANK

WILLOUGHBY SPIT

SEWELL PT

NAVAL OPERATING BASE

LEGEND

0 - 35 per 100 ml	
36 - 70 " " "	
71 - 150 " " "	
151 - 500 " " "	
Over 500 " " "	

PIG PT

STREETLA CR

HOFFLER CR

CRANEY I

ELIZABETH RIVER

LAFAYETTE RIVER

NORFOLK

LAMBERT PT

THIMBLE SHOAL

FORT WOOL

Prepared by the U.S PUBLIC HEALTH SERVICE

FIGURE 11
SURVEY OF HAMPTON ROADS SHELLFISH AREAS
1949-1950
AVERAGE COLIFORM DENSITIES
HAMPTON ROADS SECTION
1934

of 101 to 500. This density extended well out over Willoughby Bank and
North in Chesapeake Bay from Fort Monroe. Except at the mouth of Mill Creek,
the waters along the north shore from Old Point Comfort to Newport News Point
had average densities in excess of 500, while at the mouths of Hampton Creek
and Indian River and along the entire Newport News waterfront the densities
exceeded 1,000. On the south shore, the waters in the Norfolk Harbor Channel
south from Sewells Point and in the Elizabeth and Lafayette Rivers all had
average coliform densities in excess of 1,000 per 100 ml.

Let us compare these conditions with those found in the same area in
1949-50 when most of the sewage was being treated by sedimentation and chlor-
ination before being discharged, remembering that in the interval the urban
population tributary to the Roads had increased 50 per cent from 280,000
(estimated) in 1934, to 460,000 in 1950.

At the time of the later survey the majority of the waters had an aver-
age density of less than 100 coliforms per 100 ml. Figure 12. The pollution
on the south side of the Roads had been so reduced that only in the Elizabeth
and Lafayette Rivers and in a restricted area at the mouth of each did the
densities exceed 500. Even in the Norfolk Harbor Channel past the Naval
Operating Base the coliform MPN averaged less than 500 per 100 ml. The only
sample point in the entire northern section that had an average density in
excess of 1,000 was in the mouth of Hampton Creek. The average coliform
MPN in the Fort Monroe area of Chesapeake Bay had declined from approximately
150 per 100 ml. in 1934 to less than 50 in 1949-50.

EFFECT ON ADEQUATE CHLORINATION ON RECEIVING WATERS

When the 1949-50 survey started in October, there was insufficient
chlorinator capacity at the Boat Harbor sewage treatment plant in Newport
News to afford adequate chlorination to the strong daytime flows. An ad-
ditional chlorinator was on order, but it was not installed and in service
until December 1.

As two of the plants (Army Base and Portsmouth) there were chlorine
contact tanks, but apparently there was insufficient turbulance to mix the
chlorine properly with the settling tank effluent before it entered these
contact tanks. At the Boat Harbor and Lambert Point plants there were no
contact tanks and reliance was placed on the theoretical detention period
in the submerged outfalls to provide the necessary contact time.

The operating personnel of the Hampton Roads Sanitation District be-
lieved that adequate mixing was not being obtained in the outfall sewers
at Boat Harbor and Lambert Point plants and that better control of the
chlorine residual could be obtained by applying the chlorine ahead of the
settling tanks. This change in procedure was made in the plants of the
Sanitation District by December 1 and in the Portsmouth plant on January
25.

In November, arrangements were made with the Hampton Roads Sanitation
District Commission to have bacteriological samples of the chlorinated
effluents taken at each of their plants at 2:00 A.M., 4:00 A.M, and 6:00
A.M., and at 10:00 A.M., 12:00 Noon and 2:00 P.M. These samples were com-
posited in the laboratory into two samples representing the week and strong

FIGURE 12
SURVEY OF HAMPTON ROADS SHELLFISH AREAS
1949 — 1950
AVERAGE COLIFORM DENSITIES
HAMPTON ROADS SECTION

Prepared by the U.S PUBLIC HEALTH SERVICE
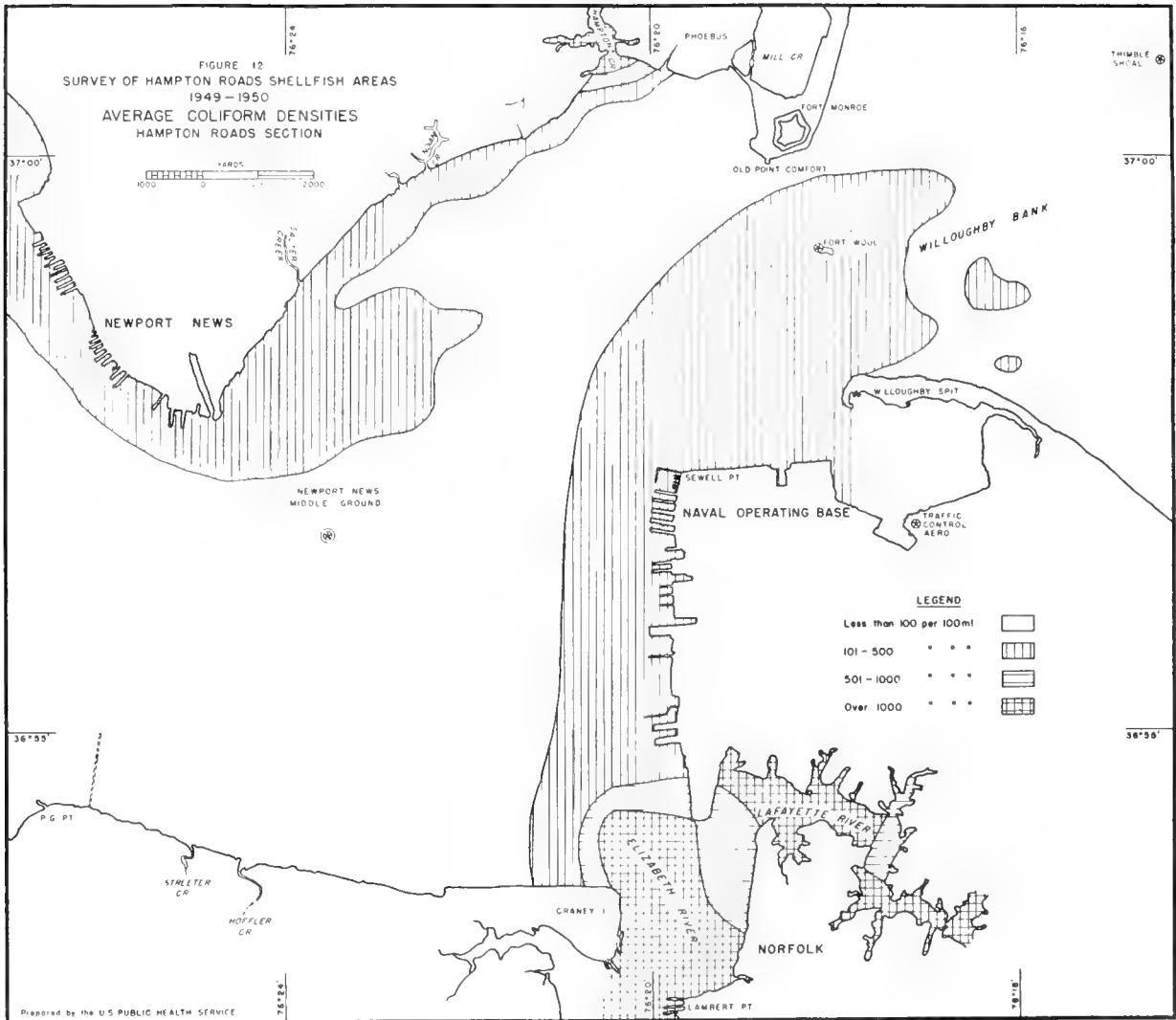
flows of the day and coliform determinations were made on the composited samples. Similar samples were taken at the Portsmouth plant. The results from these samples showed a general improvement in chlorination efficiency after December 1. This was particularly noticeable at the Boat Harbor plant which had an effluent median coliform MPN of 1,600,000 per 100 ml. prior to December 1 and less than 30 after that date.

Because of the difference in chlorination efficiency, the results of the routine water samples were divided chronologically into two periods, prior and subsequent to December 1. The medians for each sample point were determined for each period and coliform density curves were plotted.
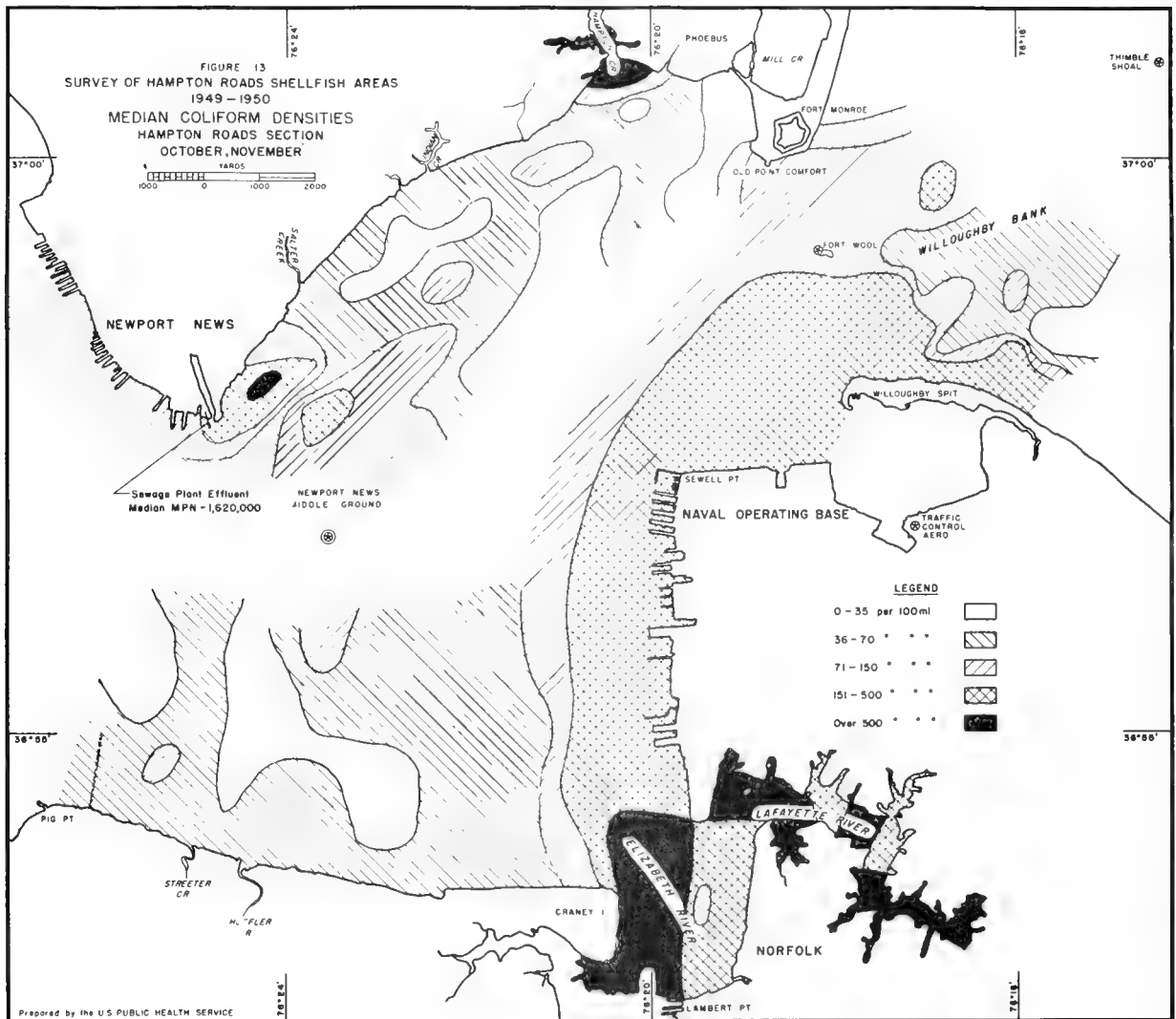
Figure 13 shows the water quality found in the Hampton Roads section during October and November when the Boat Harbor plant had a median coliform MPN of 1,600,000 per 100 ml. It will be noted that the southwest end of Hampton Flats showed relatively high pollution and that the pollution from the Elizabeth and Lafayette Rivers extended north along the Norfolk Harbor Channel and around Willoughby Spit to Willoughby Bank.
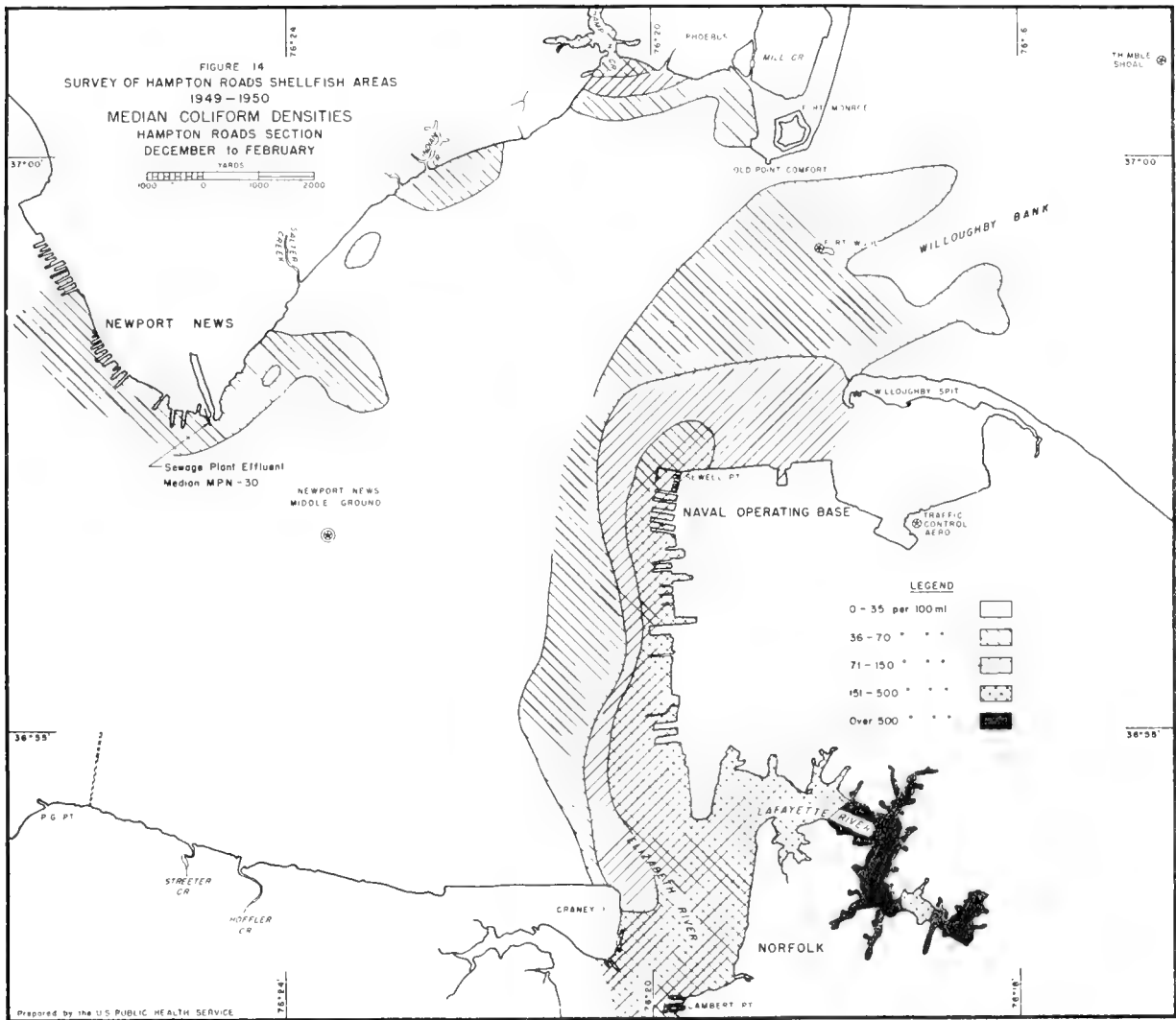
Figure 14 shows the conditions in the same area after December 1. Hampton Flats is shown to have been greatly improved. In fact, only one sample point had a median value over 70 and that did not exceed 150. It is believed that the improvement in the Craney Island Flats area is correlated with the improved effluent from the Boat Harbor plant and the improved conditions on Hampton Flats. On ebb tide a protion of the Boat Harbor plant effluent is carried through a depression northwest of Hampton Bar and over the flats, but the larger portion passes down the main channel toward the entracne to the Roads. On flood tide this diluted effluent in the main channle is pushed back upstream and floods over both Hampton and Craney Island Flats. It will also be noted that in December and January the band of heavily polluted water in the Norfolk Harbor Channel is much narrower than during the previous period and does not extend much beyond Sewell Point at the north end of the Naval Operating Base.

EFFECT OF SURVEY ON OYSTER GROWING INDUSTRY

Consideration of the water quality found in the various sections of the Hampton Roads area during this survey led to the recommendation that consideration be given to reopening for direct taking of market oysters certain section of the oyster growing beds. Such reopening would be subject to the maintenance of certain conditions in the naval anchorages and continuation of satisfactory sewage treatment. The areas recommended for consideration were as follows:

1. Hampton Flats - The central portion of the flats from about 1,000 yards offshore to the deep water channel. This area covers 1,470 acres.

2. Willoughby Bank - A triangular section west of the existing restriction line, with its base on the ship channel and its apex on the existing restriction line 1,000 yards off Willoughby Point, with an area of 1,300 acres.

FIGURE 13
SURVEY OF HAMPTON ROADS SHELLFISH AREAS
1949 – 1950
MEDIAN COLIFORM DENSITIES
HAMPTON ROADS SECTION
OCTOBER, NOVEMBER

LEGEND
0 – 35 per 100ml
36 – 70 " " "
71 – 150 " " "
151 – 500 " " "
Over 500 " " "

FIGURE 14
SURVEY OF HAMPTON ROADS SHELLFISH AREAS
1949 - 1950
MEDIAN COLIFORM DENSITIES
HAMPTON ROADS SECTION
DECEMBER to FEBRUARY

3. <u>Craney Island Flats</u> - The portion of the flats from the existing restriction line (800 yards east of Hoffler Creek) to a line about 1,000 yards west of the Norfolk Harbor Channel. This portion of the flats has an area of 4,100 acres.

Thus this survey indicated that, as a result of the collection and treatment of municipal sewage in the Hampton Roads area, a total of 6,800 acres or 10 square miles of previously restricted shellfish growing areas might be released for the taking of oysters for direct marketing.

BEHAVIOR OF OYSTERS IN WATER OF LOW SALINITIES

Victor L. Loosanoff
U. S. Fish and Wildlife Service, Milford, Connecticut

INTRODUCTION

A student of the biology of oysters will be duly impressed with the long list of references either entirely devoted to or mentioning some aspects of the behavior or condition of oysters in water of reduced salinities. A review of the literature on the biology of oysters by Baughman (1947) mentions approximately 95 such titles. The articles appearing since the publication of this bibliography and those which were not included in it would increase the list of titles to well over one hundred. Yet, as any critical reviewer would conclude, the majority of these articles deal either with isolated field observations on salinity or the data offered in them are so incomplete that they are of little value to the oyster physiologist or ecologist. A few articles, such as those of Yamazaki (1929,1932), Seno, Hidemi, Hori and Kusakabe (1926), Amemiya (1926, 1928), A. E. Hopkins (1936) and others, although greatly contributing to the understanding of the behavior of oysters in different salinities, still leave too many voids in our knowledge of this field.

How little we actually know about the physiological effects of low salinities upon the American oyster, _Crassostrea virginica_, is shown by our inability to answer satisfactorily the following questions:

1. What is the lowest salinity at which our oysters can exist normally, i.e., feed, grow and propagate?

2. What are the approximate periods during which oysters can survive in salinities lower than the minimum needed for normal existence; are these periods affected by the temperature and, if so, to what extent?

3. Do oysters of all ages require the same salinity conditions?

4. Do oysters originating in basins where salinity is low require a lower salinity to survive than oysters from basins of high and steady salinity?

5. How does exposure to low salinities, during different periods of the annual cycle, affect the ability of oysters to develop gonads and to spawn?

6. How are rate of pumping and condition of oysters affected if oysters are transplanted from normal sea water to that of low salinity or vice versa, and is there a difference if such changes are made gradually instead of abruptly?

7. What happens to shell and body liquids of oysters during tidal cycles within which the salinity of the surrounding water markedly changes?

These are a few of the questions that were to be answered when we began working on salinity, but eventually we found ourselves working on over 20 different aspects of this interesting and important problem. This report will discuss briefly some of these aspects.

In presenting this paper I wish to acknowledge my sincere thanks to my assistant Miss Phyllis Smith for her help and suggestions during these studies.

METHODS AND MATERIALS

As a study of the literature will reveal, observations on effects of salinities upon aquatic animals were usually carried on in aquaria filled with water of the desired salinity. Because of the difficulties involved in preparing new batches of water the contents of the aquaria were seldom changed. Obviously, by using such a system the animals were not kept at the optimum condition.

In our studies, on the contrary, the oysters were always kept in running water of the required salinities. This was easily achieved by using the apparatus we designed for mixing in desired proportions fresh and salt water (loosanoff and Smith, 1950a). The oysters were kept in large but shallow trays so as to provide a rapid exchange of water guaranteeing a rapid removal of metabolic products. As a rule, a continuous, measured flow of plankton was added to the running water in required quantities to assure the experimental animals enough food.

Most of our observations were made on Long Island Sound oysters accustomed to a salinity of about 27.0 p.p.t. These oysters came from extremely steady ecological surroundings where the salinity of the water practically does not change during the year. Such oysters, therefore, could be taken as the standard for the comparative studies in preference to oysters originating in water of continuously and sharply changing salinity. Furthermore, Long Island Sound oysters are known to be relatively free of such diseases as cuased by Nematopsis, Dermocystidium or Hexamita, a fact of significant importance in evaluating the results of experiments and deciding whether the oysters dies from reduced salinity or from a heavy infection of parasites, as may be the case of oysters from some areas of the gulf of Mexico which are, at times, so emaciated that they cannot be regarded as normal.

For special problems, such as comparing the salinity requirements of oysters from Long Island Sound with those of oysters originating in a less salty environment, groups of oysters were imported from such places as the upper part of Chesapeake Bay and kept, until used in our experiments, in a salinity equal to that from which they came.

All salinities discussed in this report were accurate within $\neq$ 1.0 p.p.t.

MORTALITY

Long Island Sound oysters were brought to the laboratory, separated into groups of 50, and placed directly in fresh water, 3.0 p.p.t., 5.0 p.p.t., 7.5 p.p.t., 10.0 p.p.t., 12.0 p.p.t., 15.0 p.p.t., and the control, the salinity of which was approximately 27.0 p.p.t. The latter salinity will be referred to in this paper as normal. The oysters were adults measuring between three and four inches in length. Their conditions were checked daily, attention being paid to feeding, formation of true and pseudo faces, tonus of the muscle, growth, etc. An oyster was considered dead when it was gaping and did not react when its mantle was touched with a sharp needle.

The experiments were conducted under different temperature conditions. The first series was conducted when the temperature of the water was relatively high ranging between 23.0 and 27.0°C. (Table 1). Within this temperature range a quick mortality of oysters took place in fresh water and in 3.0 p.p.t. Mortality was also high in 5.0 p.p.t., although approximately 25 percent of the oysters did survive in this salinity for a period of 30 days. However, in 7.5 p.p.t. the

Table 1. Daily mortality of oysters exposed to fresh water, water of low salinities and to normal salinity of the control at high temperatures ranging from 23.0 to 27.0°C. Sample in each salinity consisted of 50 adult Long Island Sound oysters.

Days of Exposure	Fresh Water	3.0 p.p.t.	5.0 p.p.t.	7.5 p.p.t.	10.0 p.p.t.	12.0 p.p.t.	15.0 p.p.t.	Control 27.0 p.p.t.
1								
2								
3	1		1					
4	2		1					
5	10	2	3					
6	12	7	4		1			
7	6	6	11					
8	5	9	2					
9	6	8	7					
10	4	7	2					
11	1	4	1					
12	1	2	1					
13	2	1	1					
14		1	1		1	1		
15		3	1					
16			1					
17								1
18								
19								
20								
21								
22				1				
23			1			1		
24								
25				1	1			
26								
27			1			1		
28								
29								
30								
Total Mortality	50	50	38	2	3	3	0	1

Table 2. Daily mortality of oysters exposed to fresh water, water of low salinities and to normal salinity of the control at medium temperatures ranging from 17.0 to 20.0°C. Sample in each salinity consisted of 50 adult Long Island Sound oysters.

Days of Exposure	Fresh Water	3.0 p.p.t.	5.0 p.p.t.	Control 27.0 p.p.t.
1				
2				
3				
4	1			
5	1	2	3	
6	2	2	3	
7	2	3	2	
8	8	8	2	
9	6	3		
10	5	4		
11	3	2		
12	3	2		
13	4	3	1	
14	1			1
15		3		
16	5	2		
17		2		
18	2	2		
19	2	1	1	
20		2	1	
21			1	
22		2	1	
23	1			
24		1	1	
25				
26		1		
27				
28		1		
29		1	1	
30			2	
Total Mortality	46	47	19	1

Table 3. Daily mortality of oysters exposed to fresh water, water of low salinities and to normal salinity of the control at medium-low temperatures ranging from 13.0 to 16.0°C. Sample in each salinity consisted of 50 adult Long Island Sound oysters.

Days of Exposure	Fresh Water	3.0 p.p.t.	5.0 p.p.t.	Control 27.0 p.p.t.
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11	1			
12				
13				
14				
15				
16	1	1		
17			1	
18		1		
19				
20				
21				
22	3		1	
23	1		1	
24	1		3	
25	1	4	1	
26				
27	4			
28	1			
29	2	3	2	
30	2	3	1	
Total Mortality	17	12	10	0

Table 4. Daily mortality of oysters exposed to fresh water, water of
low salinities and to normal salinity of the control at low
temperatures ranging from 8.0 to 12.0°C. Sample in each
salinity consisted of 50 adult Long Island Sound oyster.

Days of Exposure	Fresh Water	3.0 p.p.t.	5.0 p.p.t.	Control 27.0 p.p.t.
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17	1			
18				
19				
20			1	
21				
22				
23				
24				
25				
26				
27		1		
28	1			
29				
30				
Total Mortality	2	1	1	0

Table 5. Total number of dead oysters at the end of 30-day exposure
at different temperatures to fresh water, water of low
salinities and to normal salinity of the control. Sample
in each salinity consisted of 50 adult Long Island Sound
oysters.

Temperatures $^{\circ}C.$	SALINITIES			
	Fresh Water	3.0 p.p.t.	5.0 p.p.t.	Control 27.0 p.p.t.
8.0-12.0	2	1	1	0
13.0-16.0	17	12	10	0
17.0-20.0	46	47	19	1
23.0-27.0	50	50	38	1

mortality was much lower; only two animals died within 30 days. The same low mortality was recorded for higher salinities (Table 1.).

When the experiments were repeated at lower temperatures, ranging between 17.0 and 20.0°C. (Table 2), the results were somewhat different in that some oysters lived through the 30-day period, both in fresh water and in 3.0 p.p.t., and more of those kept in 5.0 p.p.t. survived at this temperature than had survived at the temperature range 23.0-27.0°C. Furthermore, although the mortality in these three groups was still high, the oysters, in general, lived considerably longer than in the first experiment, which was conducted at a higher temperature. For example, in the first experiment all the oysters kept in fresh water died within 13 days, while in the second, the last dead oyster was recorded on the 23rd day and, at the end of the experiment, there were still four oysters alive.

The next experiment conducted at still a lower temperature, ranging from 13.0 to 16.0°C., again showed that the rate of survival of oysters became higher as the temperature decreased (Table 3). Finally, at a temperature just a few degrees above hibernation the mortality in all the groups was negligible amounting to one or two individuals per sample of 50 (Table 4).

The results of these experiments, which are summarized in Table 5, indicate that in speaking of mortality of oysters in relation to low salinities reference should always be made to the temperatures existing at the time of exposure. In general, the rate of mortality of oysters, subjected to fresh water and to lower salinities, increases with an increase in temperature. For example, in 3.0 p.p.t. only one oyster out of 50 died within 30 days at a temperature ranging from 8.0 to 12.0°C., while at the higher temperature, ranging between 23.0 and 27.0°C., all the oysters died within the short period of 15 days.

Some of the oysters exposed to fresh water or a salinity of 3.0 p.p.t. at low temperatures survived under those conditions for as long as 70 and 115 days respectively (Loosanoff, 1948). Obviously, the oysters were able to survive only because during the entire period of exposure they either kept their shells closed or prevented the entrance of water of low salinity into their shells by special efforts of the mantle.

Even after being closed for such long periods some of the oysters still possessed meats that were normal histologically. In others, however, signs of tissue starvation became evident and this condition was, apparently, accompanied by the process of bacteriological decomposition. The meats of oysters of this type were usually milky yellow in color, their tissue, especially the gills disintegrating and emitting a foul odor. Regardless of this their mantles were still able to react if touched with a sharp needle.

The rather unusual phenomenon of seeing fungus, Saprolegnia, growing on the bills of the oysters was often observed in low salinities, Frequently it was also found that the oysters surviving in such salinities for long periods were building a second shell inside the original, as an additional barrier against unfavorable surroundings.

In one of the experiments, designed to determine whether oysters with damaged shells would become easier victims of low salinity, the shell edges were broken off in such a way that there was an opening permitting contact of the oyster body with the surrounding water. These oysters were placed in water of low salinity simultaneously with control groups consisting of normal oysters with

uninjured shells. At the end of long periods of exposure, sometimes lasting 30 days, the mortality among the oysters with broken shells was usually no higher than of the controls, although Saprolegnia was often growing on the areas of the mantle which the oysters pressed against the opening, to prevent entrance of water of low salinity into the interior of the shell.

In oysters kept in fresh water or in low salinities, where feeding was impossible, the crystalline style disappeared. However, regeneration of the style, upon return to normal conditions, was extremely rapid. For example, the oysters kept in fresh water for 20 days and then returned directly to water of normal salinity maintained at a temperature of about 25.0°C., where they opened and began to feed immediately, formed crystalline style. approximately 1.0 mm. in diameter, within an hour or two after the transfer.

During the entire periods of exposure to fresh water and a salinity of 3.0 p.p.t. the oyster retained the feces and pseudo feces within their shells.

RESISTANCE TO LOW SALINITIES OF OYSTERS OF DIFFERENT AGES

Several of our experiments were designed to determine whether oysters of different age groups differ in their ability to survive in water of low salinity. Oyster spat approximately four months old and 4-year-old oysters were placed in fresh water, in water of low salinities of 3.0 and 5.0 p.p.t. and in control of 27.0 p.p.t. The young and old oysters were examined daily. The experiment was repeated several times each time using 50 spat and 50 adult oysters.

The results of one of these experiments, which is representative for the series, and which was carried for 36 days at a temperature ranging between 12.0 and 17.0°C., showed that young oysters, regardless of their much smaller size, may resist unfavorable salinities as successfully as adult oysters.

FEEDING

Oysters taken from normal salinity of 27.0 p.p.t. and placed in reduced salinity may, for a period of a few hours, cease to feed even in such high salinities as 15.0 p.p.t. This, probably, can be ascribed largely to the general disturbance which accompanied the transfer. Within a day or so after the transfer, however, at least half the oysters placed in a salinity of 7.5 p.p.t. or higher were feeding. Among the oysters transferred to 5.0 p.p.t. some were also feeding within 24 hours, provided the temperature was not too high. No feeding, however, was ever observed in the oysters kept in a salinity of about 3.0 p.p.t. or lower.

Among the feeding groups the oysters kept in 5.0 p.p.t. usually exhibited strong abnormalities. For example, their feces were often white or greenish in color, composed principally of blood cells, and the fecal ribbons themselves were usually thinner than in normal oysters. Nevertheless, regardless of these abnormalities, the oysters were forming not only true feces but also pseudo feces, thus indicating that their feeding apparatus was not disturbed to such an extent that it could not function at all.

In some experiments we found indications that as the experiment progressed, the oysters of 5.0 p.p.t. grew more accustomed to their new environment and, therefore, more of them were feeding. However, in all instances it was clear that the feeding behavior of the oysters kept in 5.0 p.p.t. was somewhat different from that of the oysters of other groups kept at higher salinities.

Table 6. Mean increase in length of oysters kept in water of different
salinities for 30 days. All samples originally contained 30
adult oysters.

OYSTERS	SALINITIES IN P.P.T.				
	5.0	7.5	10.0	12.0	27.0
First Experiment					
Number survived	28	30	28	29	30
Number grew	1	18	22	26	28
Mean increase in mm.	Traces	1.5	3.5	5.3	6.9
Second Experiment					
Number survived	25	30	30	30	30
Number grew	0	21	28	28	29
Mean increase in mm.	0	2.4	6.4	9.3	8.8

GROWTH

In the course of the studies it was noticed that the rate of growth of oysters was different in the different salinities. To observe and to record the growth under such conditions groups of 30 oysters were placed in fresh water and in running water of salinities of 3.0, 5.0, 7.5, 10.0, 12.0, and 27.0 p.p.t. at a temperature near 13.0°C. Before placed in the trays each oyster was individually numbered, the edges of the shells were filed off to make new growth more easily discernable, and the oysters were measured. At the end of 30 days the oysters were examined and re-measured.

The results of two such experiments are given in Table 6. Since the surviving oysters in fresh water and 3.0 p.p.t. showed no growth, they are not included in this table. Of all the oysters that survived in 5.0 p.p.t. only one showed traces of new shell, a transparent, brittle band of material which was formed towards the end of the experiment. In 7.5 p.p.t. approximately two-thirds of the oysters grew but the increase in shell growth was significantly smaller than in any of the higher salinities. However, the increase in length of the oysters kept in 12.0 p.p.t. was not significantly different from the control.

In general, the experiment on growth of oysters kept at different salinities for prolonged periods showed once more that although oysters are able to survive in a salinity of only 5.0 p.p.t., these conditions are not favorable for normal existence.

GONAD DEVELOPMENT AND SPAWNING

The next, and perhaps one of the most important inquiries on the behavior of oysters in low salinities, is that on gonad development and spawning under such conditions. Some field data on this subject were recently collected by Butler (1949) in his studies of the conditions in upper Chesapeake Bay.

In our experiments we approached the problem from four differnt aspects. The first series of experiments was designed to ascertain what would happen to the development of the gonads if the oysters were kept for prolonged periods, late in winter or early in the spring, when they still hibernate, in low salinities and then transferred back to more salty sea water.

The next approach consisted in imitating, in its braad features, the condition existing in nature where late spring and early summer floods considerably lower the salinity over oyster beds during the entire period of normal gametogenesis and past the time of the spawning temperature. It was based on the experiments consisting in taking oysters from Long Island Sound, soon after the end of hibernation, and placing them in running fresh water and salinities of 3.0, 5.0, 7.5, 10.0 or 12.0 p.p.t. and keeping them there until spawning temperatures were reached. These studies began late in April and continued into June with fresh water and 3.0 p.p.t. oysters, and into July with oysters kept in higher salinities. The conditions of the oysters were ascertained by histological studies of the samples taken from each group at weekly or bi-weekly intervals. The initial samples taken at the beginning of the experiment showed that the gonads of the oysters, which came from water of a temperature of about 8.0°C., were normal for that time of the year, i.e., still being undeveloped resembling winter gonads.

The last group of oysters from fresh water was examined after an exposure of 51 days. None of them developed normal gonads and practically in all instances development was arrested probably by the time the oysters were placed in fresh

water. In the oysters that remained closed there still was no sign of tissue starvation and they still contained large quantities of glycogen-laden connective cells. However, in those oysters that were gaping the effects of rapid osmotic change were reflected in abnormal tissues. In some oysters an unusually large number of leucocytes was noticed, but it was found later that this condition was not generally associated with long exposures to low salinity.

The completeness of the picture of inability of oysters to develop gonads in fresh water was somewhat marred because of finding one specimen that had well developed, although somewhat abnormal, spermaries containing a large number of spermatozoa. It is quite probable, however, that this was one of those individuals that went through more extensive autumn spermatogenesis than the other males and which entered hibernation already containing a large quantity of spermatozoa (Loosanoff, 1942).

Examination of the remnants of the group in 3.0 p.p.t. showed them to be in practically the same condition as the oysters kept in fresh water.

Final examination of the oysters from 5.0 p.p.t. also showed either arrested or depressed gametogenesis. At least 50 percent possessed gonads resembling winter condition. In more advanced cases, however, the males contained secondary spermatocytes but no spermatids, while in the females only small, undeveloped ovocytes were found.

The oysters in higher salinities, including that of the control, were kept under observation for 66 days. The final examination was made on June 28, when the water temperature was already over $20.0^{\circ}C$. In the group that came from 7.5 p.p.t. there were individuals with spermaries containing ripe spermatozoa, while some females, although displaying definite abnormalities contained, nevertheless, some ripe eggs.

Oysters from 10.0 and 12.0 p.p.t. reached ripeness and some of them partly spawned. However, both groups were less advanced than the oysters from the control where the majority of the males and females were half or more than half spawned.

These experiments indicated that oysters are capable of forming gametes in a salinity of only 7.5 p.p.t. They are not able to do so in water of 5.0 p.p.t. Therefore, the dividing line should lie somewhere between these two values.

The next step was to determine what would happen to oysters with about half ripe gonads, approximately three weeks from the time of spawning, if the beds of these oysters were suddenly covered by water of low salinity. For this, oysters were again brought from Long Island Sound, at the beginning of June, and placed in running fresh water and water of salinities of 3.0, 5.0, 7.5, 10.0, 12.0 and 27.0 p.p.t. The oysters from fresh water were examined at the end of the 17th day of exposure. At that time the mortality among them was acquiring larg proportions and it was impractical to keep them longer. Histological studies showed that none of them were reaching ripe condition. In the most advanced females the gonads contained young, unripe ova, while in the males few spermatozoa could be found. Apparently gametogenesis, as in the oysters of the previous experiments, was arrested soon after they were placed in fresh water. The same can be said for the oysters kept in a salinity of 3.0 p.p.t. for 23 days.

Oysters kept in 5.0 p.p.t. for the period of 34 days, at the end of which the temperature was reaching $22.0^{\circ}C$, showed that this salinity was still too low

for the development of normal gonads. This was especially true of the females because practically none were found with well developed ovocytes. In males, however, a slow and depressed development may continue even under these conditions, resulting sometimes in the production of a few apparently normal sperm.

A marked difference was found again between the oysters kept at 5.0 and 7.5 p.p.t. The latter salinity was apparently high enough to permit normal development of cells of both sexes. The oysters kept in 10.0 and 12.0 p.p.t. also developed normally although their gonadal layer appeared to be somewhat thinner than that of the control oysters.

During this experiment the oysters spawned lightly in 7.5 p.p.t. and heavily in all higher salinities.

The final step was to determine what would happen to oysters with gonads near ripeness if they were suddenly exposed for prolonged periods to fresh water or water of lower salinity. To accomplish this oysters that were almost ready to spawn were dredged in Long Island Sound, during the last week of June, and placed in fresh water and in the salinities generally used in our experiments. The temperature at the beginning of this experiment was 20.0 ^{o}C. and soon started to show a rapid increase.

Because of the heavy mortality and gaping of ousters the experiments in fresh water and in 3.0 p.p.t. were discontinued after the 11th and 14th days respectively. Histological study showed that the oysters from fresh water suffered disintegration of ovocytes, probably caused by changes in osmotic pressure. Oysters kept in 3.0 p.p.t. that did not gape at the end of the exposure still had normal gonads, while those that were gaping were affected in the same way as the oysters of the fresh water group.

Some oysters kept in 5.0 p.p.t. gaped and became swollen soon after the beginning of the exposure. When examined two weeks after the beginning of the experiment some of them showed disintegrating eggs. Non-gaping oysters, however, still had apparently normal gonads. In our recent experiments, the results of which have not been reported as yet, the oysters kept in this salinity spawned. Such spawnings usually were feeble and although fertilization occurred the development of the embryos, as a rule, did not progress far.

All other groups placed in higher salinities were kept for 49 days. During this period spawning was observed in all groups. Nevertheless, spawning of the oysters in 7.5 p.p.t. was usually less heavy than in the oysters kept in 10.0 and especially 12.0 p.p.t.

EFFECTS OF SHARP REDUCTION IN SALINITY ON RATE OF WATER PUMPING

One of the best criteria for determining changes in behavior of oysters, paralled with changes of the conditions of the environment, is to record the changes in their rate of water pumping. This approach, therefore, was used to determine responses of oysters to sharp changes in salinity. At the beginning of the experiment the oysters were kept in a salinity of approximately 27.0 p.p.t. After their rate of pumping at this salinity had been established the salinity was rapidly lowered. In these studies the decrease was made from 27.0 directly to 20.0, 15.0, 10.0, 5.0 or 3.0 p.p.t.

The decrease from 27.0 to 20.0 p.p.t. did not markedly affect the behavior of the oysters. Nevertheless, basing our conclusions on the records of about 20

oysters, the mean rate of pumping was reduced to 76 percent of that pumped in normal sea water.

The drop from 27.0 to 15.0 p.p.t. was usually more radical and sometime resulted in closure of the oyster while the mean rate of pumping for the next six hours was only 11 percent of the original.

The drop from 27.0 to 10.0 p.p.t. was strongly affecting the oysters, usually resulting in a disturbance of the shell movement and in reducing the mean rate of pumping to only about 9 percent of the original.

At 5.0 p.p.t. the change was even more pronounced, the rate of pumping being lowered to approximately 0.4 percent.

All the data given above designate the changes in the rate of pumping for a comparatively brief period, only about six hours, after the reduction in salinity. Later on oysters in all salinities, even that of 5.0 p.p.t., showed some degree of recovery, pumping at a more rapid rate than during the first six hours. Data pertaining to this phase of our work will be presented later on.

Of equal interest to us was the question of what happened to oysters if the salinity of the surrounding water was sharply changed from low to high. To answer it groups of oysters were conditioned for different periods, usually several weeks, in water of low salinities, and then transferred directly to water of a salinity of 27.0 p.p.t. In general, the oysters survived the transfer, unless they were weakened by exposure to low salinity, as usually happens after long exposure to fresh water or a salinity of 3.0 p.p.t. However, mortality in such cases was not due directly to the rapid change from low to high salinity but to the injuries to the body tissues which occurred in low salinities prior to the transfer.

In certain experiments some of the oysters kept in low salinity were transferred to high salinity directly, while the paralled group, or groups, was brought to the same high salinity gradually, by passing through several intermediate solutions. The mortality in both groups was low, and no significant difference between the groups was noted.

In some cases the transfer from the low salinity of approximately 10.0 p.p.t. to that of 20.0 or 25.0 p.p.t. resulted in a reduction of the rate of pumping. Usually, however, a return to the normal rate of pumping was noted within an hour or two after the transfer.

It is remarkable that after being kept in fresh water or in a salinity of only 3.0 p.p.t. for as long as 60 days the oysters would soon open their shells and begin to feed and expel truw feces within the first day or even within a few hours after the transfer. The crystalline style, which disappears during the long siege of starvation, was often regenerated within two hours after the beginning of feeding. In the spring, as has already been reported, the oyster transferred to high salinity after a long exposure to low salinity proceeded with normal development of gonads.

In general, the observations indicate that oysters are capable of withstanding sharp changes from low to high salinity without experiencing serious physiological injuries.

CHANGES IN BEHAVIOR OF OYSTERS DURING TIDAL CYCLE

After learning some of the facts concerning the effects of sharp changes from high to low and from low to high salinity upon oysters it was interesting, of course, to study the behavior of oysters and to determine certain changes in their blood and shell liquids during the tidal cycle. For this study a special apparatus was designed to imitate the gradual changes in salinity of the water occurring in nature during a complete tidal cycle. The apparatus has already been described in detail (Loodanoff and Smith, 1950b) and, therefore, will not be discussed fully again. It will be sufficient to mention that by using this apparatus we were able to simulate the changes taking place in intertidal basins, such as Milford Harbor, which have a considerable inflow of fresh water during low water stages.

The chief task of the apparatus was to reduce gradually, within a six-hour period, as if during ebb tide, the salinity of the water to that of almost fresh water, and then, during the following six hours, as on the flood, gradually increase it to the full salinity usually prevailing during high water stages. The apparatus is versatile and its range of salinity could be arranged as desired.

Using this apparatus it was found that during a decrease in salinity, as on ebb tide, oysters taken directly from Long Island Sound, where salinity is approximately 27.0 p.p.t., stopped pumping at the average salinity of about 12.0 p.p.t. They often closed their shells before the salinity was reduced to 8.0 p.p.t. and remained closed while the salinity continued to decrease further until the water became fresh (Table 7).

TABLE 7. Salinities in parts per thousand at which control oysters and oysters conditioned to lower salinities cease or resume pumping and close or open their shells.

OYSTERS	SHELLS		PUMPING	
	Close	Open	Cease	Resume
Control 27.0 p.p.t.	8.0	12.0	12.0	13.5
Conditioned to 10.0 p.p.t.	2.1	4.5	4.0	5.0
Conditioned to 7.5 p.p.t.	1.1	2.5	3.0	3.5

When, as on flood tide, the salinity began to increase gradually, the majority of the oysters opened their shells when the salinity reached approximately 12.0 p.p.t. and began pumping again at about 13.5 p.p.t. As usual, wide variations in the behavior of different individuals were observed and, therefore, the figures given in Table 7 are merely means.

Oysters conditioned in the laboratory for about three months in a salinity of 10.0 p.p.t. or 7.5 p.p.t. pumped and opened their shells at a much lower salinity

than the control (Table 7). Thus, as these experiments have shown, oysters can get accustomed to a much lower salinity than the one in which they were grown.

Our experiments in which the tidal apparatus was used, and which were supported by observations on oysters taken directly from Milford Harbor at half-hour intervals throughout the complete tidal cycles, have shown that as long as the oysters remain open the changes in salinity of their shell and body fluids closely follow the changes in salinity of the surrounding water. In general, the chlorides of the sea water remained somewhat higher than those of the shell fluid, while the body fluid of the oysters showed the lowest salt content.

SUMMARY

1. The length of survival of oysters in fresh water and low salinities is controlled by temperature, being higher at low temperatures.

2. There was no significant difference in the rate of survival in a salinity of 5.0 p.p.t. or less between oyster spat and adult oysters.

3. Oysters were feeding in a salinity of 5.0 p.p.t. or higher although in the former the feeding behavior was often abnormal.

4. No growth was observed among the oysters kept in salinities lower than 5.0 p.p.t., and even in 5.0 p.p.t. virtually no growth occurred. In 7.5 p.p.t. the oysters grew but the rate of their growth was slower then in higher salinities.

5. Gonad development ans spawning of oysters in different salinities and during different periods of their annual cycle are discussed.

6. The lowest salinity at which normal development of gonads may proceed is near 7.5 p.p.t., but oysters with ripe gonads subjected to lower salinities may spawn in a salinity of only 5.0 p.p.t.

7. A sharp reduction in salinity from 27.0 p.p.t. directly to 20.0, 15.0 10.0 and 5.0 p.p.t. resulted in a decrease in the rate of pumping of 24.0, 89.0, 91.0 and 99.6 percent respectively for a period of approximately six hours after the transfer.

8. Oysters are capable of withstanding sharp changes from low to high salinity withour experiencing serious injuries. They fed, re-formed crystalline style and expelled true feces and pseudo feces within a few hours after the transfer.

9. Oysters conditioned to live in lower salinities cease or resume pumping of water and close or open their shells at lower salinity concentrations than oysters living in water of higher salinities.

10. Changes in salinity of oyster shell and body fluids closely follow changes in salinity of the surrounding water. In general, the chlorides of the sea water remained somewhat higher than those of the shell fluid, while the body fluid showed the losest salt content.

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General Character of Plankton Organisms in
Waters Overlying Shellfish-producing-grounds

by
James B. Lackey, George Vander Borgh, Jr. and Joseph B. Glancy

INRODUCTION

Most of the shellfish harvested by man come from inshore, more or less pro-
tected waters. Exceptions are abalone, certain clams and scallops. Even these
come from shallow (neritic) waters. The bottoms from which such shellfish are
taken vary considerably - mud, sand, gravel, rock. Compared to open ocean (pelagic)
waters, the waters overlying shellfish also vary a great deal, especially in
plankton. Much of this variation has to do with land-contributed material, a
rich nutrient supply for microscopic plants (phytoplankton), and for some micro-
scopic animals (zooplankton). The latter also eat the phytoplankton.

Presumably these two plankton groups, and their counterparts in the bottom-
water interface, constitute the food of the harvestable (adult) oysters, clams
and scallops. As of the present there if extremely little knowledge of what act-
ually constitutes shellfish food. In some cases a definite all inclusive intake
of the surrounding water is known, but even in these cases it is not known what
intaken material is swallowed and digested. It would appear that no shellfish
producer could efficiently manage his grounds unless he possessed such knowledge.
Certainly the dry land farmer - corn, cotton, cattle, ducks - has a scientific
knowledge of what his crops eat or require, and it is expressed in common, non-
technical terms.

This paper is an attempt to present some information on the kind and abun-
dance of plankton organisms present in waters overlying shellfish-producing-grounds
and non-shellfish-producing grounds. It is not assumed that the absence or pre-
sence of certain species or abundances is the sole determining factor for the
presence of absence of shellfish. Presumably the same plankton organisms would
be present in a given body of water whether shellfish were there or not. Then too,
these are field observations, and as such, without controls. Nevertheless it is
worth the effort to seek correlations.

The work outlined herein has been done in the following places:

The Hole, and the Eel Pond, Woods Hole, Mass.

Long Island Sound, Milford.

Gardiners and Peconic Bays, L. I.

Great South Bay, L. I.

Patuxent River, Solomons, Md.

Chesapeake Bay, Solomons, Md.

St. Mary's River, St. Mary's City, Md.

METHODS USED IN THIS SURVEY

Generally, one of two procedures is followed. A grab sample is taken,
usually 800 or 400 ml. If preservation is needed, 3 mls. of commercial formalin
per 100 mls. of sample are added, a data slip put in the jar or on it, and it is

stored, in the darkness, until tabulated.

If unkilled samples are desired, the grab sample is kept in the shade, and as cool as possible, until examined. Storage in an ice box is desirable.

For examination the catch is sedimented by settling, or centrifuging at 2500 r.p.m. for 5 minutes. The sedimented catch is treated as so many drops, usually 12.5 or 25.0, to 100 mls. of sample. This permits examination of one drop (~ 8 or 4 mls.) under a 25 mm. square, No. 1, cover glass. At magnifications of 100 or 440 diameters there are 16 or 64 paths across such a cover, and counting the organisms, then estimating the number per ml., or per liter, is simple. This method is very fast.

GENERAL PLANKTON SITUATION

Approximately 300 species of plankton organisms have been identified from the localities named. Dinoflagellate species are most numerous, followed by diatoms, then ciliates. Many genera cannot easily be split into species on counting or when sparingly represented. The diatom Rhizosolenia and the dinoflagellate Gymnodinium illustrate this difficulty. There are quite a few species also, which appear to be new or not as yet described. A number of bottom forms, swept into suspension by the currents, are commonly found in the plankton. In any given sample of 100 mls., however, there are rarely more than thirty or thirty-five genera, and perhaps forty to fifty species, which occur as frequently as once per ml. There is, however, a standing crop of from 100 to several thousand countable organisms per ml. of water. This means that any quart of water can be assumed to have at least 100,000 organisms, and often a great many more, at any time. In several hundred samples examined since 1948 there have been practically no exceptions to this statement, which is very much on the conservative side. It must be understood that this applies to inshore waters, protected bays and shallow areas generally. The open waters of the high seas may have much smaller numbers, although counts of Georges Bank waters show very high numbers.

The plankton is usually markedly different if one compares inshore and offshore waters. Decreased salinity, more foodstuff, and other conditions tend to produce a particular species aggregate, roughly intermediate between fresh water and that of the high seas. There are species which may be common to all three habitats, and quite a number are both neritic or estuarine and pelagic. Generally these ubiquitous species do not occur in as great numbers as do the more restricted species. Also, certain limiting factors, especially if present for a long period of time, tend to produce "dominant" sapcies which are characteristic of local conditions.

Now and then some plankton species suddenly undergoes a period of rapid reproduction, until it occurs in huge numbers in its area of growth. It is then termed a bloom. No satisfactory definition of a bloom is available. Usually color or turbidity is the visible evidence. In working on the Wisconsin lakes, the number 500 per ml. was arbitrarily used as defining a bloom. The smaller the species, the greater the number required to constitute a bloom, however. Blooms usually sharply cut down the numbers of other species and individuals in an area. Ary area richly supplied with food usually sees a succession of blooms, one species blooming after another, during the year.

AVAILABLE SHELLFISH FOOD

The density of shellfish populations scarcely needs comment. Any one who has seen mussel beds exposed at low tide, or an oyster bar in shallow, clear

water, and who knows the rate of growth of shellfish, can appreciate their food requirements, at least as to volume. Turner notes as many as 500 soft clams, almost 3/4 or an inch in length, per square foot in certain Barnstable Harbor areas. Since shellfish are largely sedentary, their food must come to them, and they manifestly eat very small organisms. On the bottom there is potentially available a constantly renewed crop of protozoa and algae in the mud-water interface. In the summer of 1934 a study of the waters at Woods Hole showed a list of some 264 species of microorganisms exclusive of diatoms, and of these 264 only 79 were found in bottom samples. An attempt was made to estimate the rate of accumulation of bottom populations in this area and it was found that in 24 hours the bottom of a Syracuse dish would acquire 70,600 microorganisms (exclusive of bacteria and other organisms than algae and protozoa) per square centimeter. Nevertheless, this is probably not an adequate food crop for a dense shellfish population.

The plankton is available from top to bottom, and currents must inevitably bring it to the region of the incurrent siphon of a feeding clam. Let us recall that in several hundred plankton samples examined since 1948, the lowest number of organisms (this one over the oyster reef known as Thomas Bar at Solomons, Md.) was over 300,000 for any liter of water there; and the highest number well over 300,000,000. It appears that shellfish beds in the waters studied are literally blanketed by food which is replacable at a high reproductive rate.

<center>LOCAL DIFFERENCES IN PLANKTON
COMPOSITION AS RELATED TO SHELLFISH PRODUCTION</center>

The waters around Woods Hole produce few shellfish, although mussel beds formerly carpeted certain areas, and clams are fairly abundant. The plankton content of the Woods Hole waters is high. Great South Bay at Greens Point formerly produced great quantities of oysters, and the Bay still contains great numbers of clams. Its plankton also is high, much more so than the Woods Hole waters, even if we exclude the small green alga referred to hereafter. Are the differences significant for shellfish production?

Diatoms occur more frequently at Woods Hole, and more species are found there. There is considerable opinion and actual observation that diatoms are a favorite or good oyster food. In this connection it should be noted that at Woods Hole the diatoms are frequently the larger species, and that they attained bloom proportions (500 per ml.) in only 10 samples out of thirty, and that only 5 of these 10 were during the months oysters would be feeding. At Greens Point diatom blooms occurred in 14 out of 24 samples, but only 6 during feeding temperatures. The most abundant Greens Point diatom in these 6 blooms was Chaetoceras solitaria, a very small form. Skeletonema was almost absent during feeding temperatures in Great South Bay.

No bloom of dinoflagellates occurred at Woods Hole among these samples, and only three at Greens Point. The species varied widely in both localities. Generally, species occurrence was parallel in both places except that Prorocentrum micans occurred much more frequently at Woods Hole, and Prorocentrum tri ulatum much more abundantly at Green Point. Ciliate species were more numerous and numbers more abundant at Woods Hole; this was a surprise, because it was felt Great South Bay had a high bacterial population, and ciliates feed on bacteria. Total ciliate numbers never exceeded 125 per ml., and were usually only a few.

There were conspicuous differences in the small flagellates, however. At Greens Point all species of Chrysophyceae occurred 70 times, and at Woods Hole

32. Chrysococcus cingulum occurred in all of the Greens Point samples; 10
times in bloom proportions, its maximum being 4,450 per ml., Nov. 18, 1950.
It occurred but 13 times in the Woods Hole samples, but never in bloom pro-
portions. Its maximum being 87 per ml., May 13, 1949. Practically the same
thing was true of Rhodomonas, but its numbers were always smaller; and a color-
less saprophytic organism tentatively named Chilomonas marina was also closely
parallel in behavior. At Woods Hole, the small colorless monad flagellates often
termed protozoa usually occur in the mud-water interface, and their occurrence
in the plankton is about one-third as frequent as at Greens Point. At the latter
place they also occur at times as blooms. This may be important, since they are
eaters of bacteria to some extent, and may partly supplant the ciliates.

The importance of this is that Great South Bay contains, as its present
flora and fauna, an abundance of supposed good shellfish food, yet the oysters
there are so poor as to be useless. This statement might be extended to cover
the final group, the green plankton algae, not supposed to be important in the seas.
One of these small green flagellates, Pyramimonas, occurs abundantly at Greens
Point, as frequently, but far less abundantly, at Woods Hole.

There remains one other great biotic difference between the two situations.
The "small form" of uncertain taxonomic status, which occurs so abundantly in
Great South Bay is lacking in Woods Hole, or any other waters studied in this
survey. It attained 500 per ml. at Greens Point in March, 1950; rose to 2,234,422
per ml. October 28, 1950; and finally in December went back to 700 per ml., then
disappeared. This, incidentally, has been its history for years. Instead of
there being a succession of different blooms, this one remains all the warm months,
as a dominant, and only a few other organisms as Chaetoceras solitaria, Chrysococcus
cingulum and Oicomonas can bloom at all during these months. Most of the other
blooms at Greens Point come in the winter.

Time does not permit treatment of the other areas, but mention can be made
of their overlying plankton. Gardiner's and Peconic Bays are excellent oyster
grounds. They have no "small form." Instead, their plankton is varied, and is
frequently dominated by the diatoms Skeletonema, Chaetoceras debilis, and occasion-
ally Thalassiosira. But their total numbers of individuals, except rarely, are
less than that of Great South Bay, and more nearly like Woods Hole as to species
list and abundance. However, they do have enormous blooms at times; a species of
Cochliodinium reddened square miles of water in 1951. There are far less small
flagellates of the three groups considered above.

St. Mary's River - really a wide bay - off the lower Potomac River, is an
excellent setting ground, but poor for fattening oysters. There is a good crop
near its mouth, however. Any fertilization is largely of non-human origin - some
farmland and some forested land surround it. There is a fairly large plankton
content; many species not encountered elsewhere in the survey, but which are not
too numerous. The diatoms are rather few in number, and do not include Skeletonema,
although some small Chaetoceras are fairly numerous at times. Nor is the dino-
flagellate population large. Instead, there are large numbers of several kinds
of small flagellates, and at times very small green flagellates, which may be
gametes or zoospores of algae. Altogether the situation here supports the idea of
small flagellates being good larval food, and diatoms and dinoflagellates being
a better food for adult oysters.

The other three Chesapeake stations are somewhat like Gardiner's Bay and
Peconic Bay in having a rather general, but not too large plankton. They are
decidedly lower in diatoms (lower salinities, higher temperatures) but make up for

this in somewhat more dinoflagellates, and more numerous Cryptomonas and Rhodo-monas.

Altogether, it seems probable that any inshore water can provide a suffi-ciently large plankton content to maintain a large and thriving shellfish popula-tion. There is some evidence that preferred foods are diatoms and dinoflagellates for adult shellfish and that relatively small plankton populations are best. It is also evident that it is kind, and not abundance which is critical; and that for the larval stages, the smaller plankton flagellates are best.

SHELL GROWTH VERSUS MEAT YIELD IN THE OYSTER C. VIRGINICA

Philip A. Butler, Research Biologist
U. S. Shellfishery Laboratory
Pensacola, Florida

The process of growth is extremely complex and the yardsticks devised by biologists to measure it are indeed many. In oysters, it is expecially difficult to study since the body is hidden by the valves. Measurement of growth is further complicated by the failure of the oyster to reach a definitive size. So far as I know, in the absence of enemies, the oyster grows on and on. Nor can we use sexual activity as a criterion of growth maturity since in warm waters the oyster may spawn when a half inch in diameter and in cold waters the first spawning period may be delayed until the animal is three inches long. Biologists as well as fishermen have been prone to accept length as a valid criterion for growth in oysters, and certainly it is the easiest. Our state laws regulating oyster harvesting have followed suit and the three inch "market" oyster is more or less standard up and down the coast. However, since the amount of growth in an oyster is directly related to the profit in harvesting, we find that there are some who do not use the three inch measure to determine when oysters shall be harvested. We find, for example, in the Gulf area, the commercial canner does not buy his oysters depending on how long they are but rather on how many cans he will obtain from a barrel. And in other areas, we find the private planter depending on how many pints he can shuck from a bushel and not on whether the oysters will pass the legal size limitation. The phenomenon of increase in shell length is an interesting one in itself and its study is profitable, but its bearing on the growth of the oyster, i.e. the production of meat, is far from direct. I do not suggest that this has gone unrecognized in the past, but there have been many reports of rapid increases in shell length which, with or without the writer's intention, have been interpreted as meaning rapid meat production. The reading public has been educated to consider these two items as synonymous.

After several thousands of measurements made on oysters at Pensacola, I have realized that our data on seasonal changes in lengths of oysters were not giving us all of the information possible to aid in understanding the complexity of oyster growth in southern waters. I should like to summarize very briefly the results of some of the observations which have influenced my thinking on this matter.

a. The following data were selected from a typical growth experiment in which the oysters were maintained under as normal conditions as possible consistent with the required examinations.

In the four month period between June and October in each of three successive years, average increases in length in this population, were as follows:

1st year	16 to 34 mms.
2nd year	57 to 59 mms.
3rd year	68 to 68 mms.

During the third summer when there was no increase in average length, average weight increased from 94 to 105 gms. and average volume increased from 49 to 54 mls.

Obviously during these four months of the third year the oyster was growing even though there were no changes in the average length of the group. It might be

noted here, for comparison, that during this same period in Long Island Sound, Loosanoff has reported that in mature oysters 70% of the total length increase for the year takes place.

b. In this second group of oysters, controls remained continuously in the water while an experimental group was exposed daily to simulate tidal effects. The average percentage increases in length, width and weight in the two groups are shown for the first six months of the year:

	Controls	Experimentals
Length	32%	0%
Width	39%	0%
Weight	160%	100%

c. In the following experiment, oysters which had been growing under crowded conditions on shell cultch were separated and the following percentage increases were obtained at the end of a six month period:

Length 1.5%   Width 0%   Volume 40%

At the end of the next six month period, percentage increases were:

Length 22%   Width 25%   Volume 233%

The following measurements of two oysters in this group show individual extremes:

Length -1.5   Width -2   Volume ⫫ 168
        -2   ⫫3   ⫫ 125

These decreases in total length for the year were not the result of breakage but true examples of retrogressive changes in usable shell area, which are perhaps more common than is suspected.

It is readily seen, then, that under a variety of conditions, we may observe essentially no changes in length or width while impressive changes are taking place in weight and volume, in other words, when the oyster is growing. Moreover, and I shall not indicate any data at this point, we find that in Florida at least, increases in weight and volume take place, more or less regularly, during every single month of the year in contrast to the interruption of changes in length and width which may occur in various seasons or following various experimental treatments. The regularity of changes in volume during the growing months as compared to changes in length and width has also been recorded by Loosanoff in Connecticut. And I suspect that it is characteristic of the growth of this oyster wherever it is found.

Now, the value of using volume as a yardstick in measuring growth is, I think, apparent but you run into serious difficulties if it is used to assay the productive potential of a given reef which you may have opportunity to examine but once. The reasons for this are obvious if you consider the striking differences found in the character of the shell. In areas having high infestations with boring sponge and clams, the shells may be massive and yet contain very little meat; conversely, in areas of rapid growth, with the production of very thin shells and relatively small oysters, the meat yield may be quite high. Even in a given area, population densities and the type of cultch radically influence the thickness of

the shell and correspondingly affect the relationship of total oyster volume to meat yield. We have considerable data illustrating these differences but I can do no better than to quote some figures of Moore's on experimental plantings he made in Louisiana in the period 1906-09:

Age	Average Size	No. per Bushel	Pt. Yield	Conditions
32 months	$4\frac{1}{2}$	240	$3\frac{1}{2}$	Crowded on oyster shell
33 months	4	150	7	Not crowded on oyster cultch
33 months	$3\frac{1}{4}$	175	7	Not crowded on clam cultch

These data illustrate briefly the fact that even in the same area at the same time the total length and volume of the oyster is not necessarily an index of the meat yield. You are familiar with these conditions and I need not elaborate on them. By shucking a few random samples from a reef during the harvesting season, the meat yield can be quickly determined at any particular time and one population can be compared with another. It is, however, a different matter for the investigator who may wish to determine the potential yield of a certain area or of a certain type of culture technique. He has to sample oysters both in season and out, when they are spawning and when they may be hibernating--under these conditions the yield of meat obtained from a sample may have little bearing on what those oysters will produce under the optimum conditions found at harvest time.

I should like to cite here some data recently obtained to illustrate this point. We had two groups of oysters, one growing as singles, the other group crowded on shell cultch. At ten months of age, when samples of these oysters were shucked, the yield from the single oysters was 30% greater than the yield from the crowded oysters. Two months later when the oysters were again sampled, the yield ratios had reversed, and the crowded oysters showed about 25% more meat than the singles. Superficial examination might have led to the conclusion that the crowded oysters had grown or improved that much in condition. What had actually happened, was that the single oysters had all spawned and were thin and watery while the majority of the crowded oysters were still unspawned.

So the problem arises, what yardstick can the investigator use to estimate the potential yield from an oyster population under the best conditions existing at any time in the year at some particular location?

In our experience we have found that such a yardstick exists and that it is an extremely convenient one; namely, the ratio of total volume of the oyster to the shell volume. Under poor growing conditions, where meat yield is low, this ratio approaches but of course never reaches 1.0, and as oysters improve in meat yielding ability this ratio approaches and may surpass 2.0. In other words the larger the meat containing cavity in proportion to shell, the higher the ratio. In practice, we find that our best oysters have a total volume: shell volume (ratio) of about 1.8 and our poorer oysters have a ratio of approximately 1.2. This ratio has many convenient uses and I shall indicate some of these briefly:

a. First--it reflects the volume of the oyster meat when the animal is, or was, in the peak of condition.

b. It is independent of meat condition--when gathering a large number of samples in a survey, for example, it is not necessary to be concerned about the comparable preservation of the meats; since it eliminates the factor of seasonal fluctuations in meat quality, it is equally useful in pre- or post - spawning periods as well as in the harvesting season; and it can even be used in the study

of oysters long dead.

c. It eliminates the subjective impressions gained from rapid shell growth and overall large size.

d. When used in conjunction with meat volume, it can be used to evaluate the effects of disease, pollution and semi-permanent enviromental changes.

Now, you will gain the impression that I am enthusiastic about the use of this yardstick, and I am. We have found it applicable in comparing oysters in widely different but adjacent environments, and I believe it will be equally useful in comparing oysters which are widely separated geographically.

Most of you are familiar, I am sure, with the factors which our statistical section uses in compiling data on the production of oyster meats from the different states. These conversion factors help estimate the pound yield of meat from a standard bushel of oysters, and vary from a high of about $7\frac{1}{2}$ in Connecticut to less than 3 in Florida. These factors are based on not only the quality of the meats but also on the marketing methods used in the several states and for this reason do not indicate the meat yield from comparable samples of oysters in the different areas. I have attempted to re-evaluate these factors on the basis of a bushel of culled, market size oysters and the results are tabulated below. I am indebted to many biologists, including Mr. Logie in Canada, Messrs. Glancy, Engle, Beaven and Lunz, for the data and information used in this table, and I wish to take this opportunity to thank them for their opinions and data. I wish to emphasize, too, that any faults in the interpretation of this material are entirely my own.

TABLE 1

	CANADA	LONG ISLAND SOUND	CHESAPEAKE BAY	SOUTH CAROLINA	FLORIDA
Months to produce market oysters in:					
Poor areas	84	80	60	48	48
Best areas	48	42	18	18	18
Average areas	66	52	30	30	30
Months in hibernation	5	4	3	0	$\frac{1}{4}$
Growing months to produce market oyster—average	46	36	24	30	30-
Lb. yield per bushel USFWS Statistic, 1949	-	7.6	4.5	2.62	4.13
Biologists' estimate of lb. yield per bushel	7.5	7.5	6.0	4.0	4.0
Oz. yield/calendar month	1.8	2.3	3.2	2.1	2.1
Oz. yield/growing month	2.6	3.3	4.0	2.1	2.1

I wish to point out that the data on which this table is based are quite meager. The figures presented are my interpretation of material provided me by biologists who have had many years of experience in the respective areas. I believe, that despite this reservation, there is a clear cut differential in oyster rowth in the different areas. The Chesapeake oyster produces the greatest amount f meat in unit time both from the commercial and biological point of view; oys-

ters in the warmest areas produce the least and the other regions are intermediate.

It should not be thought that these conclusions are contradicted by the reports of rapid shell growth in the Gulf area. It is true that 3 and 4 inch oysters are commonly produced in the Gulf in a year's time and that 6 inch oysters in 0 months are not rare, but it is equally true that 3 inch oysters are not infrequent in Chesapeake Bay in 6 months' time, that is at the end of the first growing season. Mr. Beaven tells me of 6 inch specimens which were only 17 months old, and this of course represents only 14 or 15 growing months. Surprise has been expressed in the literature that oysters in the Gulf may add as much as 0.3 mms. per day in shell length. But it should be noted that these observations were on oysters from a few weeks to less than a year old. Dr. Loosanoff has reported increases of 0.5 mm. per day in a 3 year old oyster, an age when growth is notably slower than in spat; and in the colder waters of Long Island Sound.

Now there are several possible explanations for what I consider the faster growth of oysters in northern waters and I should like to mention one of these:

Oysters in northern waters are harvested primarily near or during hibernation temperatures when the animal naturally has accumulated the maximum amount of food reserves. In warmer waters, where hibernation temperatures are the exception, the oyster is not obliged to store a supply of food in its tissues and during a major portion of the harvesting season its energy requirements are supplied by its daily food comsumption. Consequently, the meats are not as fat, on the average, as the meats from oysters harvested in colder waters. This is also illustrated by the situation found in some years when in the spring, water temperatures rise suddenly well above the normal level for spawning. The oysters, and this is my opinion, having little stored glycogen available for transformation into eggs, require days and even weeks to accumulate sufficient food for the maturation of the gonads.

It is of greater importance to recognize why oysters living in northern waters are able physiologically to store more food reserves than warm water animals. I believe the reasons underlying this condition justify my conclusion that northern oysters grow faster than oysters in the south. Since the rate of biological processes is dependent on temperature, one may expect a greater food requirement, for example, in warm water than in cold water. It has been found that the temperature coefficient for many biological processes, or Q10 as it is called, is approximately 2. This means that for each 10^0 rise in temperature within the tolerance levels of the animal, the speed of metabolic activity is approximately doubled. In the case of the oyster we may interpret this to mean that month by month throughout the year, the food and oxygen requirements of the Gulf oyster are one-half to twice as great as for oysters in northern waters--just for bare subsistence. Now when this greater food requirement is coupled with a probable decrease in food availability in the Gulf, it is reasonable to assume that Gulf oysters have to devote much more of their energies to the problem of existing. Although pumping and feeding rates may be similiar in the two areas, the percentage of food consumed which can be devoted to body building, i. e. to growth and to storage, is probably far greater in cold waters than in the Gulf.

In summary: The ratio of total volume to shell volume appears to possess certain advantages in estimating growth and meat yielding potential of oysters as compared to the customary dimensional measurements.

An evaluation of the growth of oysters from various areas on the basis of available data, indicates that from both the biological and commercial point of

view, oyster growth is greatest in the Chesapeake Bay area and rate of growth declines progressively in warmer and colder environments.

I believe that a survey of oyster producing areas using the total volume: shell volume (ratio) will substantiate my estimate of the growth differential in the different geographical regions.

THE HYDRAULIC CLAM RAKE, A NEW METHOD OF GATHERING SEED CLAMS

John Glude, U. S. Fish and Wildlife Service, Harlan Spear, U. S.
Fish and Wildlife Service, and Dana Wallace, Maine
Department of Sea and Shore Fisheries

Section 1 - Presented by Dana Wallace

First, I would like to present a thumbnail sketch of our State of Maine soft-shell clam fishery and why it was necessary to develop a system of digging seed clams quickly and cheaply.

Maine's soft clam industry is strictly a "between the tides" fishery, and the commercial taking of clams is in no way mechanized. The clam hoe is the digging implement.

Over the last five years we have produced an average of 7,497,590.4 pounds of shucked meats per year with from 2,016 to 3,337 diggers employed each year and with the average digger, over this period of time, taking 2.10 bushels per day and digging an average of 4.60 months out of the year during the period of low water tides. Our mean range of tide is between nine and twenty-one feet which periodically gives us thousands of acres of exposed flats.

We are wrestling with the multiple problems of management of the fishery and one of the phases that we have felt should be investigated is the matter of transplanting small clams into depleted areas. I understand that a great deal of the oyster production is dependent upon transplanted stocks.

We started investigating this type of conservation in 1946 and relayed hand-dug clams into the flats in many communities. We used small clams usually found locally in good setting but poor growing areas.

Hand digging of small clams is a slow and costly operation. Every bushel of clams that was ultimately produced by this method cost many, many times the clams' value when digging and transplanting costs were considered along with the survivals and growths of the clams.

In 1947, we were very fortunate in finding a big natural setting area with low yearly survivals but where, after a summer's set, the tiny clams usually numbered better than one thousand to the square foot. We needed many clams if we were to continue the investigation of the feasibility of commercial transplanting or have clams available in goodly numbers for further experimental work.

(Note: Kodachrome slides illustrated the following and are not reproduced here)

Western Beach is an area approximately 4,000 feet long and 2,000 feet wide with a shoreward portion strip 2,800 x 250 feet containing the available yearly sets. This beach lies on the shore of Saco Bay east and adjacent to the mouth of the Scarboro River. On the outer portion of the beach, the sand ripples badly and sets are quickly ground to bits or smothered.

Shell debris and live clams often are washed up on the shores and, although we have not seen, we have been told of windrows of clams a foot thick and several

yards wide that occasionally result after heavy storms.

The abundance of clams on the surface of the flats that have been washed from their natural burrows have been examined and photographed. Fortunately, this big set occurs in sand from which the clams may be readily screened. We used a screening method to recover the tiny clams. The sieve is $\frac{1}{4}$-inch mesh hardware cloth and, with this type of panning in a runoff stream, one man could gather nearly a bushel of clams during a five-hour low tide. With the clams running approximately 20,000 to the bushel, this was a goodly number of clams but not of commercial proportions; and it was very hard work.

We borrowed different types and sizes of pumps in order to experiment with separating the clams from the sand and tried to design gear to collect the clams.

Federal and state personnel kept modifying the digging equipment; and now I will turn the discussion over to John Glude, Chief of the Federal Clam Investigation, who has slides showing the hydraulic clam rake as well as photos of how the seed clams are being used and allied problems.

Section 2 - Presented by John B. Glude

(Slides cannot be reproduced here)

The hydraulic clam rake is based upon a simple rake which I saw used in Japan to obtain seed for private clam farms. The Japanese rake was pulled from a boat as the seed beds were below the low tide level. In Maine, where the seed beds are exposed at low tide, it was possible to work from the beach, but a water supply was needed to wash the clams from the sand.

Dana's pump combined with the rake gave us the original hydraulic clam rake. Successive modifications have resulted in the light, compact rake shown.

Water from a small runoff stream is pumped under 50 pounds pressure to the manifold on top of the rake. The jets of water cut through the soil ahead of the blade and wash the sand and clams into the rake. The speed of the rake is about 5 to 10 feet per minute.

The sand goes through the 1/4" mesh sides and bottom of the rake and the seed clams go into the mesh bag. Runners on each side of the rake can be adjusted to permit digging from 2" to 5" depth. The bag, which holds about one-half bushel, is emptied into trays about every 10 feet.

The seed clams recovered average slightly less than one inch in length. Each bushel contains about 20,000 clams. With the rake, three men get 20 bushels of seed per tide as compared to two bushels by hand. Seed clams broadcast upon the ground can dig in by themselves in a short time.

Each clam farm consisted of 8 plots of 1/100 acre each. Clams were planted at 50 or 100 per square foot. Farms planted in 1951 were located at Jonesport, Southport Island, Sagadahoc Bay on Georgetown Island, Scarborough, and Wells, Maine.

A planting with clams on the surface as the incoming tide covered the flats was found by the next low tide to have practically all of the clams burrowed into the ground.

Growth of seventy clams planted at Jonesport April 1951 was compared with seventy clams dug from this farm July 1952.

A white part of the shell was apparent which was deposited before the clams were transplanted from Scarborough to Jonesport in 1951. Poor growth exhibited by some individuals and good growth shown by others serve to demonstrate the great variation in growth among clams under identical conditions.

Growth rate of clams planted at Wells, Maine in April 1951 and dug July 1952 was observed. A 1951-52 winter check was present near the edge.

Growth of clams in the Wells, Sagadahoc Bay and Jonesport clam farms from April 1951 to 1952 has been compared. Sagadahoc Bay clams grew about 15 millimeters the first summer, Wells and Jonesport about 13 millimeters. Growth began earliest at Wells and latest at Jonesport correlating with water temperatures. Growth also terminated first at Wells and last at Jonesport so the seasonal growth was about the same. In general two growing seasons are required to produce marketable two inch clams from one inch seed.

A planting density of 50 clams per square foot and one of 100 per square foot were compared. Growth at the heavier concentration appears to be less, but the difference may not be statistically significant.

The survival of clams planted at Wells, Sagadahoc Bay and Jonesport also has been compared. Plantings at 100 per square foot and 50 per square foot were included. Other farms at Southport and Scarborough were complete failures because of predators.

The flats were frozen at Sagadahoc Bay when the seed clams were planted so many drifted off with the incoming tide. As a result, only about 25 and 50 clams per square foot became established instead of 50 and 100 as we had planned. Considering this, the survival at Sagadahoc Bay has been fairly good since the population still is about 10 per square foot.

Green crabs and boring snails of the genus Polynices ate most of the seed at Jonesport by June. Those that survived this attack showed little mortality to the present time.

At Wells, the green crab population was low in April when the seed clams were planted. The clams grew rapidly and were well established before the crabs became numerous. Gradually, however, the crabs dug out the clams until by September less than 20 clams per square foot remained.

Plantings of 50 clams per square foot resulted in a population nearly as great as plantings of 100 per square foot. This is shown by the convergence of the red lines and blue lines. We would have produced more clams by planting twice as large an area at the rate of 50 per square foot rather than planting at 100 per square foot.

As a result of these experiments we planted 50 bushels of seed in Sagadahoc Bay in May 1952 in cooperation with Maine Department Sea and Shore Fisheries. These clams were planted at 35 to 50 per square foot and have survived well to date.

Many clams demonstrate the difficulty of interpreting age and rate of growth from the rings on the shell. We know that a given clam was planted April 1951 at Jonesport, Maine and dug July 1952, but where is the winter check? From our monthly samples we know only that a narrow white band is 1952 growth. Otherwise we might think the previous line was an annulus.

Green crabs, Carcinides maenas are Maine's worst clam predators. In a laboratory experiment each crab consumed 15 seed clams in 24 hours. On the flats they destroyed our Southport clam farm in 3 weeks. Records indicate a northward extension of their range during recent warm years. Three years ago they were first noticed at Jonesport, Maine and now they are abundant there. Only last year they were first reported from New Brunswick. Even fairly large clams can be crushed and eaten by green crabs. A three inch crab was observed which had just cracked the shell of a two inch clam. Numerous holes found in the flats were dug by green crabs. The deepest was 9-1/2" so that crabs can easily dig a 2-inch clam.

The moon shell, cockle, winkle or clam borer of the genus Polinices is a serious predator wherever found. The characteristic hole it drills through the shell of the clam leaves no doubt as to the culprit's identity.

The winter Flounder Pseudopleuronectes americanus is a serious clam enemy in Canada. Dr. J. Carl Medcof reported an eleven inch flounder whose stomach contained 315 seed clams. They also nip off the siphons of adult clams causing injury and sometimes death.

The horseshoe crab Limulus polyphemus is an important clam predator, particularly in Massachusetts. Our experimental farms in Plum Island Sound and Harry Turner's plantings in Barnstable have been destroyed by horseshoe crabs. Fencing will exclude them, but is expensive.

This spring Dana Wallace found a cove at West Bath, Maine, where small hard shell clams or quahaugs occurred at a density of 300 per square foot. Since these were stunted from overcrowding and likely to freeze in a cold winter he wanted to transplant them to a lower tide level. We tried the hydraulic clam rake to see if it would operate in soft mud covered with a foot of water as well as it did in sand. A number were caught and the rake tipped on end to wash the clams into the bag.

These quahaugs now average about one inch in length and are large enough to be raked into windrows and then forked into basket for transplanting. If the set had been discovered a year ago when the clams were smaller, the hydraulic rake would have been a practical method of gathering this seed.

Radioisotope Studies of the Utilization of Dissolved Phosphorus and Calcium by the American Oyster[1]

Lawrence Pomeroy, Rutgers University

For the past two years Dr. Haskin and I have been fortunate to have the support of the U. S. Atomic Energy Commission in a study of the distribution and accumulation of radioisotopes of physiological importance in shellfish. I should like to outline briefly the scope of our project and then tell you about our results thus far on the utilization of dissolved materials by oysters.

We began by making a quite broad survey of the possible applications of radioisotope techniques to the field of phosphorus and calcium because they were known to be of great physiological importance, were among the isotopes readily available from Oak Ridge, and were isotopes possessing physical properties which made them particularly suited to tracer work. Our survey included some studies of feeding and digestion in oysters, using mass cultures of Chlorella sp. labelled with phosphorus[32]. We were able to make estimates of the efficiency of feeding and sorting of Chlorella suspensions and to determine the rate of digestion of Chlorella. Both calcium and phosphorus are important in shell deposition, and we made some small contributions to the understanding of that process. Our program has also been concerned with the utilization of materials dissolved in sea water by oysters, and I shall describe some of that work in detail.

In our studies of the utilization of radioisotopes, we have endeavored to relate our finding to public health questions whenever possible. Because the American oyster is an estuarine animal, the possibility that it may accumulate radioactive river effluents or fission products from other sources of contamination needs to be considered.

Now I should like to describe some of our findings in detail. One of our earliest experiments was to place groups of oysters in battery jars containing phosphorus[32]. It is possible to obtain phosphorus of very high specific activity, so it was possible to do this work with natural sea water without appreciably increasing its natural phosphate concentration. The total concentration of ionic phosphate was from one to two microgram atoms per liter of sea water. The mean rate of uptake of labelled phosphate by various tissues was measured after several time intervals. Slide 1. After intervals as short as one hour radioactivity is found only in the gills and in the circulating blood. In addition to the tissues shown, adductor muscle was sampled, and never showed a significant amount of radioactivity in periods up to 64 hours. The apparent drop in specific activity at 64 hours as compared with 16 hours is not significant, so indications are that a steady state is reached in these tissues within about 16 hours.

For purposes of comparsion, we prepared tissue slices of the same

series of oyster tissues and placed them in sea water containing P^{32} for similar time intervals. The results are shown in the second slide. Since smaller numbers are involved here, the actual points are shown and lines of best fit are plotted for each tissue. There is some individual variation, but in repeated experiments the lines of best fit for the various tissues always appear in the same series. It also is evident, in spite of some stray points, that here we have a straight line relationship between uptake and time, with no steady state yet reached. This suggests that in the intact organism there is some control on the uptake process, or possible that removal soon is as great as uptake.

The third slide indicates why we chose to represent the uptake in terms of specific activity rather than simply as counts per minute per unit weight of tissue, as is often done in this type of study. There are significant differences in the phosphorus content of the oyster tissues, and this must be taken into account in phosphate uptake studies.

The fourth slide shows the uptake of phosphate ions by oyster blood. Samples were taken from the pericardium, a total phosphorus determination was made on an aliquot and radioactivity counted on another. The per cent of blood phosphorus involved is very small, and a steady state is rapidly reached in the blood. This may well be a control on general uptake rate. Our data indicate that in the intact normal oyster phosphate ions are absorbed only or chiefly by the gills. The pallial curtains of the mantle, for example, have been found to wholly exclude the external medium when closed and to absorb no phosphate ions on their exposed outer surfaces. After being absorbed by the gills, phosphate ions may be incorporated in gill tissue or they may pass into the blood in which they enter the general circulation. Since the labile fraction of blood phosphate is very small, and a steady state is quickly reached, these ions are available to the other tissues only in low concentrations. It is possible that other controls may also be imposed by the tissues receiving phosphate ions from the blood. This is indicated by the consistent differences in uptake level of different tissues as indicated in the first slide.

We are not carrying our studies of this uptake process to the tissue level by means of radioautographs, using methods which retain water-soluble phosphates in the tissues and thus indicate the true distribution of recently absorbed ions. Our results by these methods indicate that for the first few hours most radioactivity is in the blood. After twelve hours radioactivity begins to appear generally distributed in the tissues, and at twenty-four hours it is still generally distributed in higher concentration. It is probable that much of the labelled phosphorus is still in tissue fluids in water-soluble form after 24 hours. However, some labelled phosphorus is incorporated into cell structure within that time. This was demonstrated by Bevelander who prepared radioautographs by the usual aqueous methods and found localization of radioactivity in the sub-epithelial region of the mantle. Since his method removed all water-soluble phosphorus compounds, his results indicated incorporation of labelled phosphorus into cell structure, presumably nucleoproteins.

We are currently working on the overturn rate of phosphorus in oysters. The fifth slide gives some indication of this. This shows the excretion of

phosphorus by three oysters. The water was sampled for phosphorus at
two or three day intervals for a month, and the points represent daily
values for a two or three day period. The initially high values represent
wastes accumulated while the oysters were in storage prior to the beginning
of observations.

The significance of the uptake of dissolved phosphorus in the overall
economy of the oyster is probably small, for the amount obtained from this
source amounts to less than one per cent of the phosphorus needs, as indi-
cated by excretion studies. This source may be of some special importance
in the gill, however; a greater amount is retained by the gills than by
other tissues, and it has been suggested by Ronkin that this phosphate may
be used in the production of adenosene triphosphate to support ciliary action.

We have studied the uptake of calcium ions in the same general way
outlined for phosphorus. Again, uptake is apparently principally by the gills,
although it has been shown by Wilbur and co-workers that isolated mantle pre-
parations will take up calcium[45] and deposit it as shell. The uptake of
calcium by the blood differs markedly from the uptake of phosphate ions.
Calcium reaches a comparatively greater concentration in the blood.

In an effort to find out if this calcium was bound to blood proteins,
we placed oysters in sea water containing calcium[45] after which we removed
samples and dialyzed them for 24 hours. After that time radioactivity was
equal on both sides of the dialysis membrane. This indicates that the cal-
cium taken recently from the water is either in simple ionic form or is in
exceedingly labile combination with blood proteins. This in an important
point with reference to shell deposition. Several workers have advanced
the hypothesis that all or part of the blood calcium of mollusks is in the
form of a calcium proteinate, and that this compound is the precursor of
shell, being precipitated by mantle enzyme systems and separating into the
protein matrix and calcium carbonate crystals. Our preliminary studies do
not lend support to this theory, although they are quite inadequate to dis-
credit it.

From our studies of the uptake of dissolved phosphorus it is evident
that the major source of phosphorus is food materials, with the gills possibly
making a significant use of dissolved phosphate ions. Calcium, however, is
probably derived largely from that dissolved in the water. It is present
there in considerably greater amounts than is phosphorus, and it is taken
up rapidly, with local concentrations developing in the mantle within a few
hours. Our studies indicate that in the oyster there is no extensive storage
of calcium in any organ comparable to that in gastropods, and that the needs
of shell deposition must be met at the time deposition occurs from the immedi-
ate environment.

The overturn of calcium in mollusks is complicated by the deposition
of shell, the removal of shell for buffering during periods of anaerobic
metabolism, abrasion and erosion by associated organisms, and by exchange
between the shell and calcium ions in sea water.

Some additional theoretical significance can be attached to our studies
of the utilization of dissolved calcium and phosphorus by oysters. The fact

that these two ions of different charge both enter by the same pathways and are widely distributed in the organism suggests that other dissolved substances may enter also. These may serve to supply metabolic needs or may in some way act as stimuli to the organism. The extent to which the many dissolved materials in sea water may be absorbed and utilized by oysters and other marine organisms is at present still unknown.. We are not attempting to answer some of these questions with further radioisotope studies.

FOODS AND FEEDING OF OYSTERS AS OBSERVED WITH THE
USE OF RADIOACTIVE PLANKTON

by

Daniel J. Floyd
Special Shellfish Investigations
U. S. Fish and Wildlife Service
Beaufort, North Carolina

The question of food and feeding of bivalve mollusks has aroused the interest of investigators from the beginning of research on these animals. There are two important theories as to the chief source of food for these ciliary feeders. One theory, supported by Petersen, Petersen and Jensen, Blegvad, Spärck, Fox, and Coe, suggests that the principal source of food is from dust-fine detritus from cells of plants and animals. The second and oldest theory is that the most important source of food is live plankton organisms. The work of Nelson, Hunt, Martin, Yonge, and Loosanoff supports this theory.

The mechanism of feeding in bivalve mollusks involves a system of ciliary tracts which not only carries food particles to the mouth but also provides for some separation of food from mud and sand. The oyster, as demonstrated by Nelson, uses both the gills and palps to accomplish this sorting. The cilia carry the large particles which land on the gills to the ventral groove where they entangle in large amounts of mucous. Ingestion of such particles is less likely than ingestion of the smaller ones which cause less mucous formation, and which the dorsal groove carries to the palps where a more intensive separation takes place. This separation is not perfect as the oyster rejects some good food material and ingests some mud and sand.

In addition to the size of the particles, their physical and chemical characteristics may cause rejection of the material. The oyster does not accept spiny organisms in general but usually accepts smooth one-cell organisms. Very little is known about the chemical sensitivity of the feeding mechanisms. Considerable quantities of food present in the water at one time tend to cause the secretion of large amounts of mucous and the oyster rejects the whole mass. Some other bivalves are able to feed readily in the presence of a concentrated supply of food.

Once food material enters the digestive tract, there is still a question as to whether or not the oyster will digest and assimilate it. In the past, investigators have based the measure of utilization on growth. A group of oysters fed a unialgal culture showing more growth than the unfed control group is positive proof that the oysters are utilizing the material. However, the use of the radioisotope tracer techinques in the study of feeding plankton to bivalves offers many advantages. If plankton organisms labeled with a radioisotope as a tracer are fed to oysters and supply the only source of that radioactive element, any radioactivity found upon analysis of the tissues is definite proof that the oyster has utilized the labeled plankton organisms. This radioactivity may appear in the organic or

inorganic state depending on the degree of utilization.

This report deals with the adoption of this radioisotopic tracer technique to the study of the food and feeding of the oyster. The work is not complete and is presented at this time primarily to show that the method of radioisotope tracers incorporated into plankton organisms is of value in such studies.

Radioactive phosphorus, P^{32}, was chosen as the tracer element to be used. Dr. Rice of our laboratory has reported on the problem of labeling plankton organisms with radiophosphorus and this subject need not be dealt with here. In general he found that he could label plankton organisms and that they held radioactivity for a long period of time. Some were more difficult to label than others, depending on the characteristics and nature of growth of each species. The cells took up and held from 95 to 99.5 percent of the radioactive phosphorus of the media, which contained a concentration of 1.01 uc/cc. of P^{32}.

The oysters for the feeding experiments were adults of the Eastern oyster, Crassostrea virginica, held in running water in outside tanks at the laboratory. Before use they were brought into the laboratory for adjustment to the laboratory conditions. Sea water, collected and aged in carboys in the laboratory until plankton-free and stable, served as the medium for suspending the plankton cells to be fed. The salinity range was 32 to 35 parts per thousand and the pH 7.9 to 8.1 The feeding experiments were carried on at room temperature.

In the first group of experiments the oysters were fed in finger bowls containing one liter of aged sea water. Tracings on a kymograph drum recorded the shell movement and the hours the oyster remained open. The oyster received three feeding of labeled cells into 50 cc. of media at five-hour intervals. This dilution gave concentrations about 25 percent of the concentration reported by Loosanoff and Engle as necessary to cause the oyster to close due to the turbidity of the media. The feces and the pseudofeces of the oyster were collected prior to each feeding and after the last feeding. Measurement of the cells not filtered by the oyster was from an aliquot of the material left in the feeding dish. After a feeding period of 24 hours, the oysters were placed in a dish of fresh aged sea water to allow time for complete digestion and assimilation. They were then opened and the tissues tested.

In the second group of experiments, in order to avoid the varying concentrations of plankton caused by the addition of 50 cc. of cells at one time, which may cause an unusual number of cells in the pseudofeces, it was decided to have uniform and continuous feeding. This was accomplished by supplying the oyster in the feeding dish with a flow of sea water and plankton cells. The concentration of cells fed was regulated by dropping a thickly populated stock culture into a mixing chamber in the sea water supply line at any desired ratio. The flow or water through the apparatus was at a very slow rate, far less than the rate that the oyster is capable of pumping, which reduced the volume of radioactive waste material. This reduced flow helped the efficiency of filtration by forcing the oyster to repump the water.

Non-radioactive plankton cells were fed until a continuous string of feces appeared. Then, over a period of three hours, 100 to 150 cc. of a medium containing active cells were fed. Non-active cells were again fed until no radioactivity appeared in the feces.

After feeding, the oysters were placed in containers of aged sea water and time allowed for completion of digestion and assimilation. The oysters were then opened, the tissues ground, and the various phosphorus-containing fractions separated. Measurement of the radioactive phosphorus content of these fractions indicated the utilization of the plankton cells fed.

The feces, pseudofeces, and an aliquot of the material left in the feeding dish were collected, wet-ashed, and measured for radioactivity as a liquid using standard radioactivity measurement techniques. From these measurements, the percentage of cells was calculated.

Assuming that the cells each take up the same amount of activity, the radioactive counts per minute represent the number of plankton cells present in the culture. If there are 1,000 plankton cells in a sample having a radioactivity of 1,000 counts per minute, then each count per minute represents one plankton cell. A sample of fecal material giving 50 counts per minute would have present 50 plankton cells labeled with radioactivity. Also, an oyster containing 100 activity counts per minute in its tissue must have digested at least 100 labeled plankton cells, since the labeled plankton cells are the only source of P^{32} available to the oyster.

A study of control oysters kept in the same medium with the plankton cells removed shows that the radioactivity of the tissues could not have been the result of direct absorption of P^{32} from the medium but must have come from the cells fed. The controls did not contain any appreciable amount of radioactivity in their tissues. The medium contained almost no radioactive phosphorus, 95 to 99.5 percent being in the plankton cells.

The species of plankton used included two diatoms, Nitzschia closterium (56 microns in length) and Skeletonema costatum, a filamentous form (5 by 9 microns in size). Three Chlorophyceae were fed. These included a species of Chlorella (1.7 by 3.3 microns in size) and two flagellated species, one of Carteria (11 by 13 microns in size) and one of Chlamydomonas (7 microns in size). One ciliated protozoan was used, Plagiocampa marina (9.5 by 25 microns in size).

It is possible to account for the radioactive plankton cells added to the container in which the oyster is fed. Such an accounting is important, for there is a tendency for the cells to settle and to stick to the bottom and sides of the container. Also, the cells remaining in suspension and not filtered by the oyster must be measured, particularly whenever the oyster receives a continuous supply of sea water and plankton.

Table 1 shows the percent of the cells settling out and not available to the oyster as food under the conditions used in these feeding experiments.

For Plagiocampa cells, 45 percent settled out in the feeding dish and 67 percent of the Carteria cells. The cells settling out for the other species amounted to about 33 percent. Of these four species, Chlamydomonas has been fed in two series of tests and each time one-third of the cells fed were recovered from the cells settling out in the feeding container.

Table 1.
Percent of cells added not available to the oyster.

Species	Repeated Feeding	Continuous Feeding
Nitzschia	35.8	
Chlamydomonas	33.5	35.2
Carteria	67.0	
Chlorella	35.5	
Skeletonema	32.2	35.7
Plagiocampa	45.1	

The acceptability of various forms by the oyster is of great interest. A comparsion of the percentage of the cells fed to the oyster and deposited by the oyster in the feces and the pseudofeces is shown in Figure 1. Of all the forms fed, Chlorella was the smallest. The oysters readily accepted the Chlorella with 33.4 percent of the cells going through the digestive tract and only 16.4 percent appearing in the pseudofeces. At the other extreme was the diatom Skeletonema, a filamentous form, of which only 6.4 percent of the cells were in the feces and 47.1 percent in the pseudofeces. The grass-green alga Carteria of which 67 percent settled out in the feeding dish, showed the least number of cells filtered by the oyster. There appeared 6.3 percent in the feces and 11.7 percent in the pseudofeces; only 18 percent of the cells fed. The oysters filtered 53.4 percent of the Skeletonema cells from the water. With the exception of the cells of Chlorella and Chlamydomonas, the greatest number of cells filtered from the water by the oyster appeared in the pseudofeces. Very few of the Chlamydomonas cells appeared in the pseudofeces.

In the experiments using continuous feeding, the oysters more readily accepted the cells fed. The number of cells appearing in the pseudofeces decreased by one-half. The number of cells in the feces doubled in the feeding of Chlamydomonas cells and tripled in the case of Skeletonema.

From the foregoing results one can learn only a little of the story of the acceptability of various forms by the oyster. Apparently size, shape, and abundance at one time are some of the controlling factors. However, it seems possible that continued studies using cells labeled with radioactive materials will give us much information.

The fact that the digestive tract of the oyster contains certain materials is only an indication that the oyster may assimilate and utilize them. Following the fate of the radioactive phosphorus of the plankton cells ingested is an excellent means of ascertaining if these cells are broken down and the phosphorus absorbed and assimilated. Since the only source of radioactive phosphorus is that of the phosphorus compounds of the cells entering the tract and not appearing in the feces, radioactive phosphorus in the metabolic pool of phosphorus and entering into the phosphorylated compounds of the oyster tissues can only mean digestion and absorption of the materials of these particular cells.
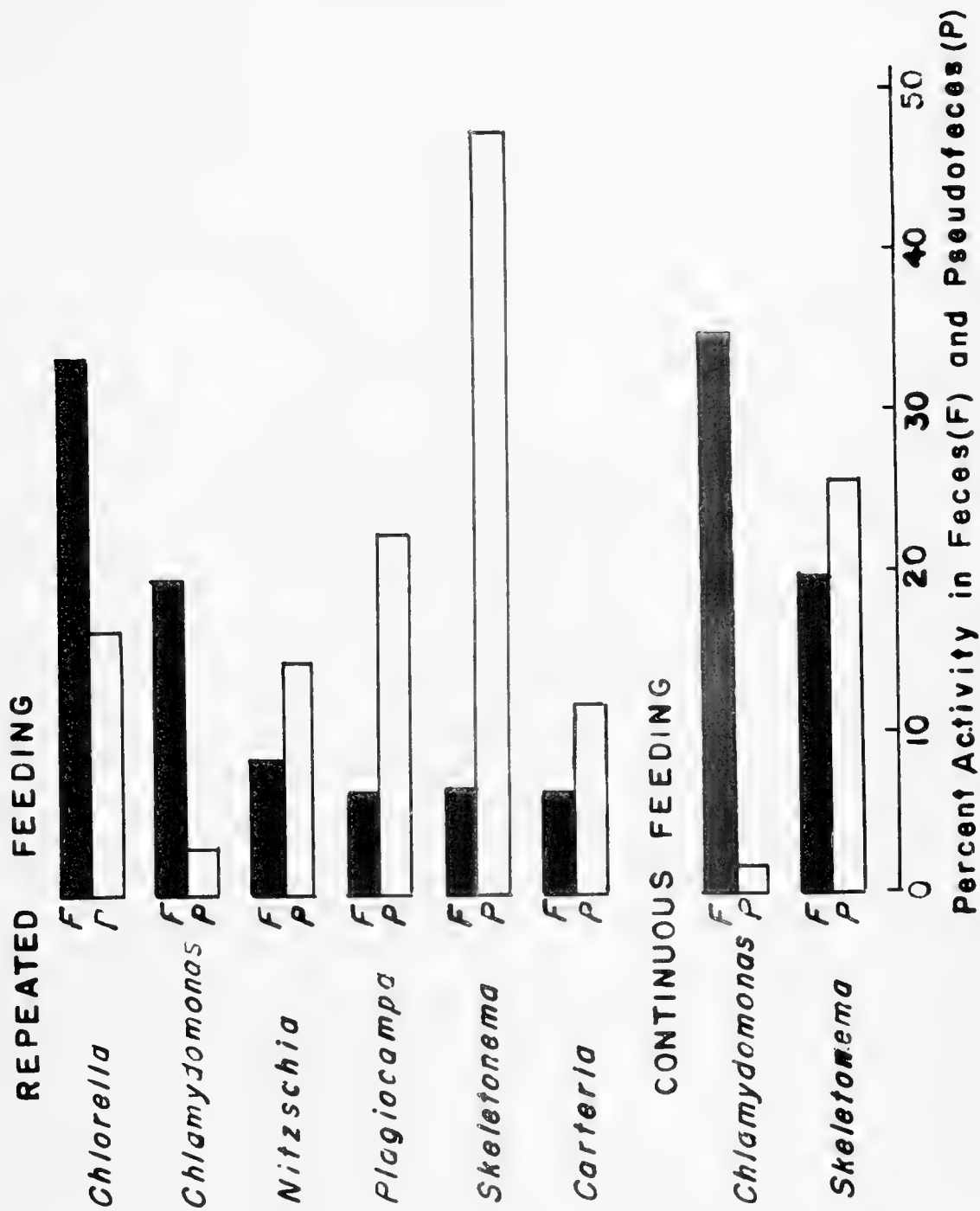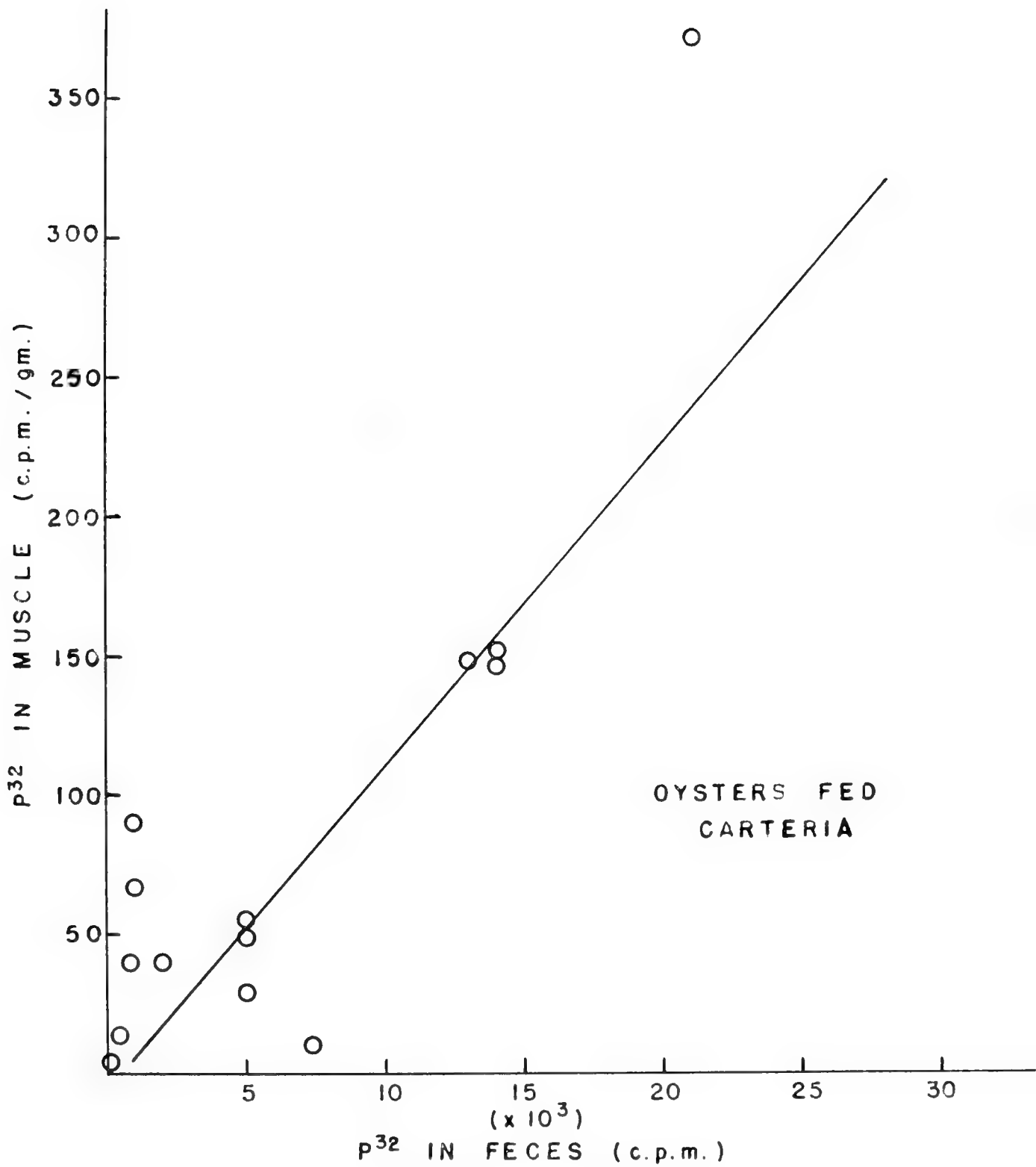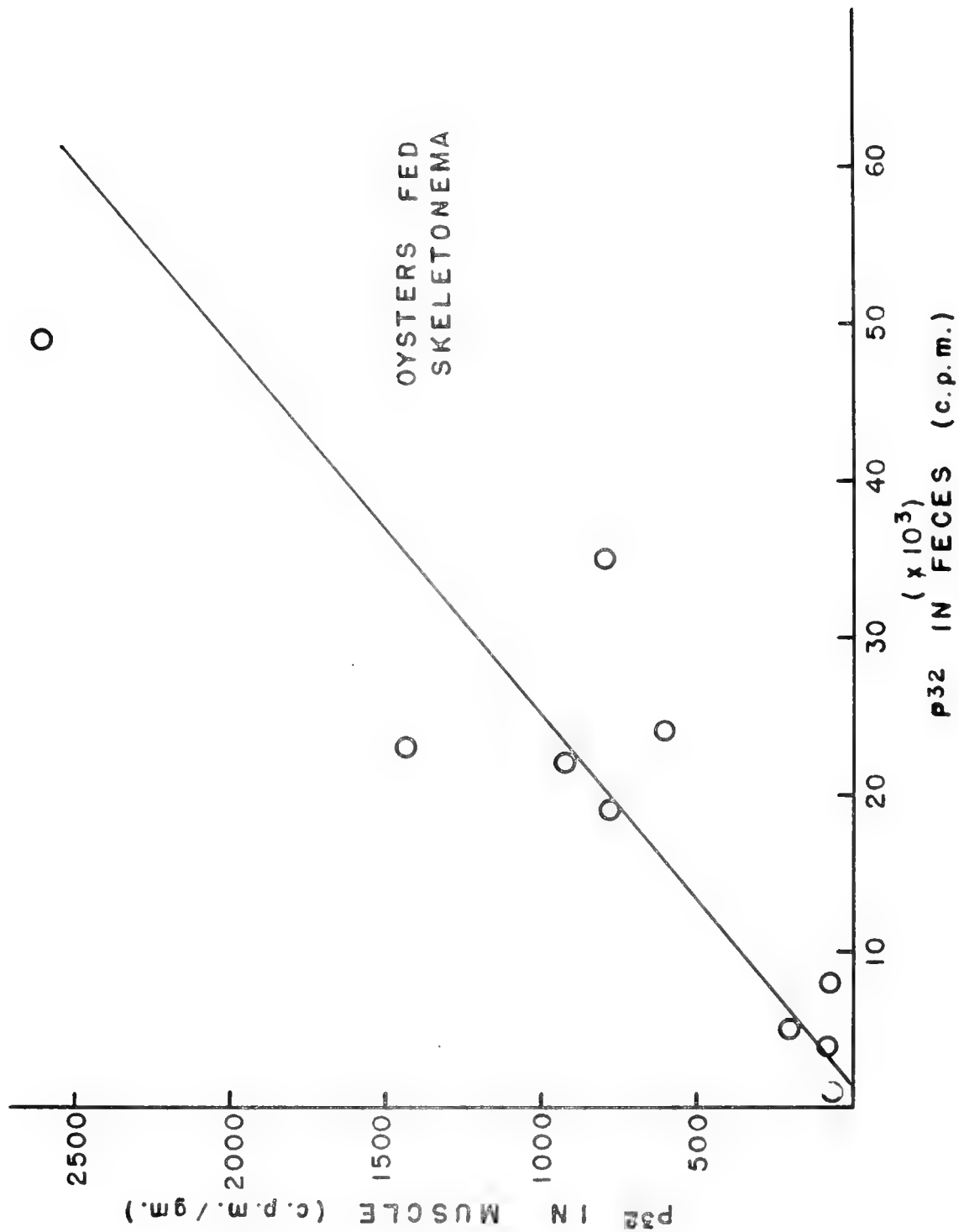
FIGURE 1

FIGURE 2.

OYSTERS FED
SKELETONEMA

FIGURE 3

OYSTERS FED
CHLAMYDOMONAS

P32 IN MUSCLE (c.p.m./gm.)

P32 IN FECES (c.p.m.)
(x 10³)

FIGURE 4

OYSTERS FED
CHLORELLA
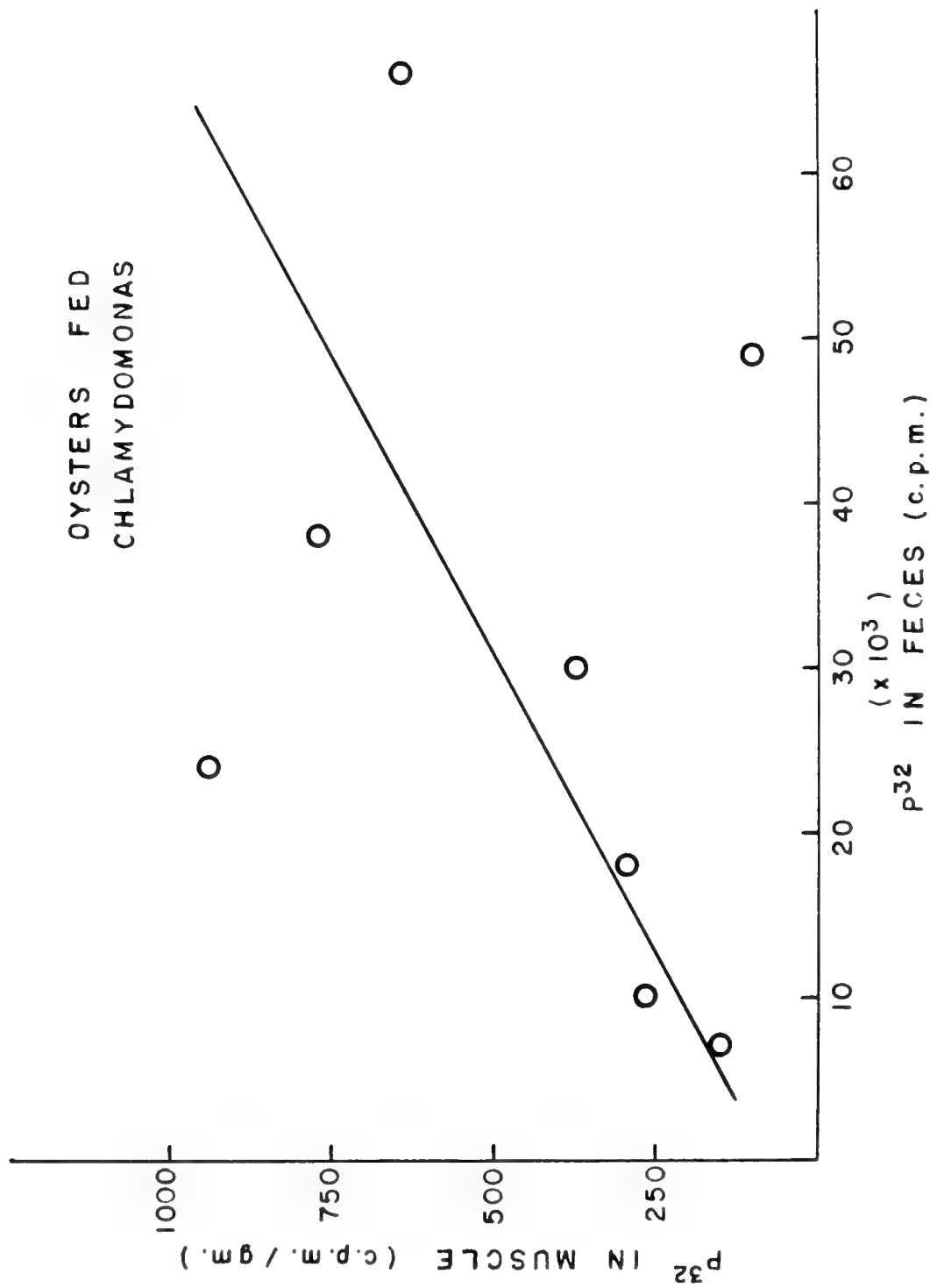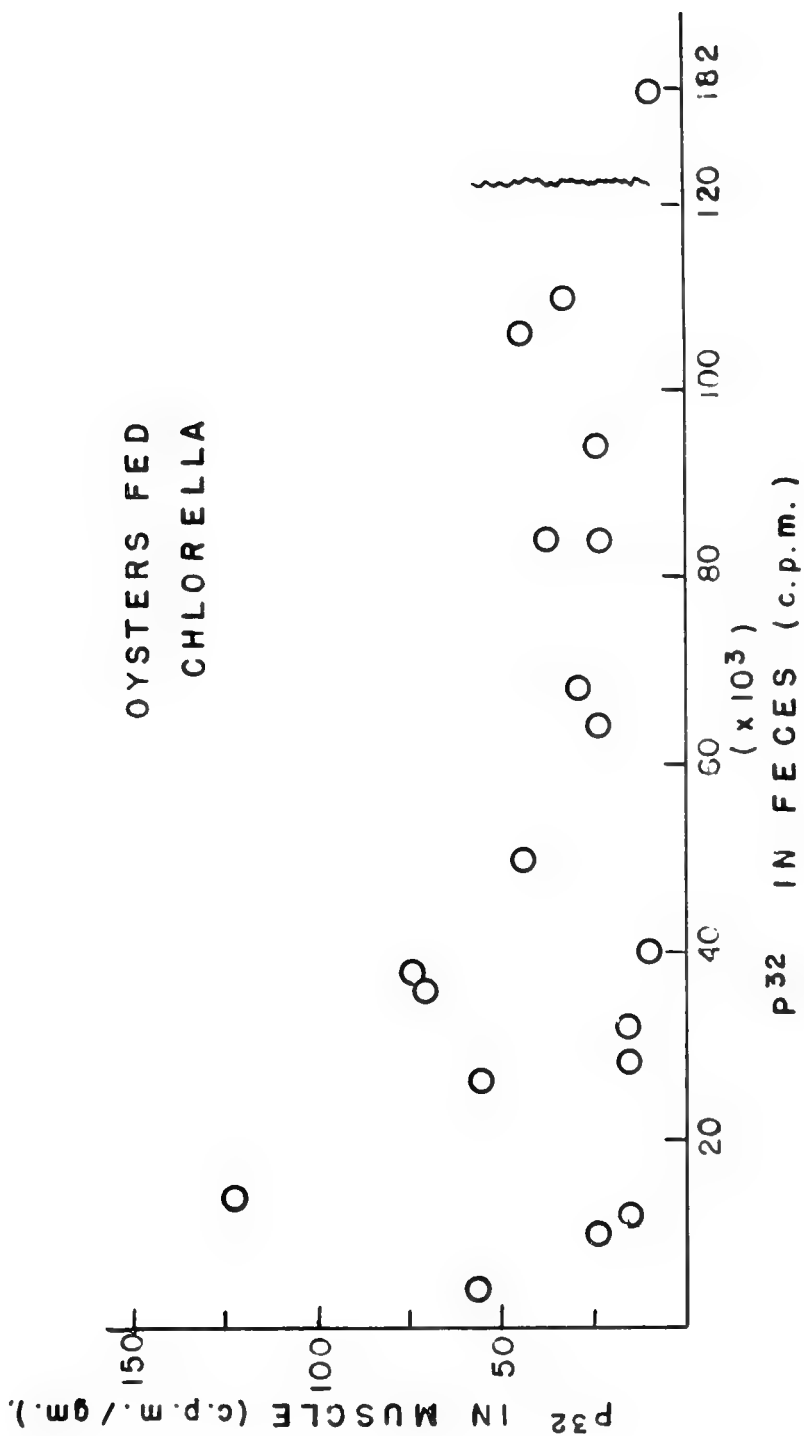
FIGURE 5

One might expect a positive correlation between the number of cells passing through the intestinal tract and the amount of nutrient material assimilated by the cells of the oyster tissues if digestion and utilization of this ingested material occurs. Conversely, the lack of correlation would suggest that there was no utilization of this ingested material. Figures 2,3,4, and 5 show graphs comparing the amount of radioactivity of the cells passing through the intestinal tract, the radioactivity resulting from the P^{32} they contain, and the P^{32} content of the phosphorus-containing compounds of the adductor muscle of the oysters after the feeding period. Figures 2, 3, and 4 show that there is good correlation between the number of radioactive cells of Carteria, Skeletonema, and Chlamydomonas in the gut and the radioactive phosphorus of the compounds of the adductor muscle. Some irregularities appear in the experiments using Chlamydomonas. It is believed that these resulted from the excessively high temperatures of the laboratory during these particular feeding tests. Figure 5 shows no correlation in the case of the feeding of Chlorella. Even though there were more radioactive Chlorella cells passing through the intestinal tract of some oysters, no accumulation of P^{32} took place in the phosphorus-containing compounds of the adductor muscle.

Experiments on the feeding of the ciliate protozoan Plagiocampa were not complete enough to allow the drawing of conclusions. The amount of P^{32} taken up by the cells of this form was too small to allow accurate measurements of the small amounts assimilated by the oyster with the techniques employed.

In summary, quantitative measurements of the selection and ingestion of certain possible food organisms by the oyster by the use of radioisotope methods have been made. Although the studies are incomplete, the results agree with those of previous work that the sorting of particles for ingestion by the oyster is dependent in part on size, shape, and abundance at one time. Because of the accurate quantitative measurements made possible by the use of radioactive tracers, further work will give a more complete understanding of the extent of sorting that takes place.

The data presented demonstrate digestion, absorption, and assimilation of the material of certain plankton cells fed experimentally to oysters, as well as the lack of utilization of the material of one species ingested by the oyster. The phosphorus of the plankton cells utilized by the oyster was assimilated and incorporated into the organic phosphorus-containing compounds of the oyster tissues.

FURTHER GROWTH STUDIES ON THE QUAHAUG, VENUS MERCENARIA

Harold H. Haskin
Rutgers University, New Brunswick, N. J.

At the 1949 meetings of the National Shellfisheries Association in Old Point Comfort a report was presented giving the results of the first two years studies of the growth of Venus mercenaria in various New Jersey waters. These studies have been continued and extended and results through 1950 will be summarized here.

In the 1949 paper methods were described by which, in one growing season, it was possible to construct a composite growth curve for a Venus population. The basic assumption in using such a curve is that the population will enjoy a succession of identical growth seasons. Even though this does not occur, the method is convenient in comparing growth rates for different areas and for the same area from season to season. This is illustrated in the figures which follow. All of the growth curves discussed here are calculated on the basis of planting seed quahaugs averaging 10 grams in weight
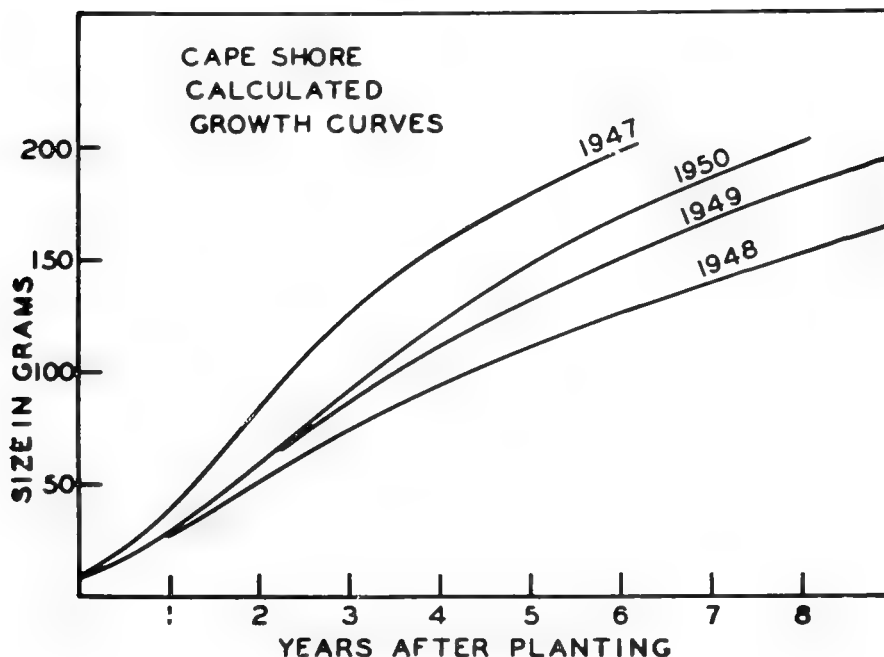

Figure 1

Figure 1 shows a series of calculated growth curves for our experimental plantings on the tide flats of Delaware Bay at our Cape Shore Laboratory. Each of these curves is bases on data for the single season indicated. It is apparent that the growth rate observed in 1947 has not since been equalled. The variation in growth rate from season to season at this location may be illustrated by listing the times required to produce "medium clams from 10 gram seed if a succession of similar growing seasons obtained:

1947 - 4 years	1949 - 6 years
1948 - 8 years	1950 - 5 years

The composite growth curve obtained by combining the data for these four seasons closely approximates the 1950 curve (figure 2). In a succession of years like the four shown, 150-gram clams would be obtained from 10-gram seed in $5\frac{1}{2}$ seasons.
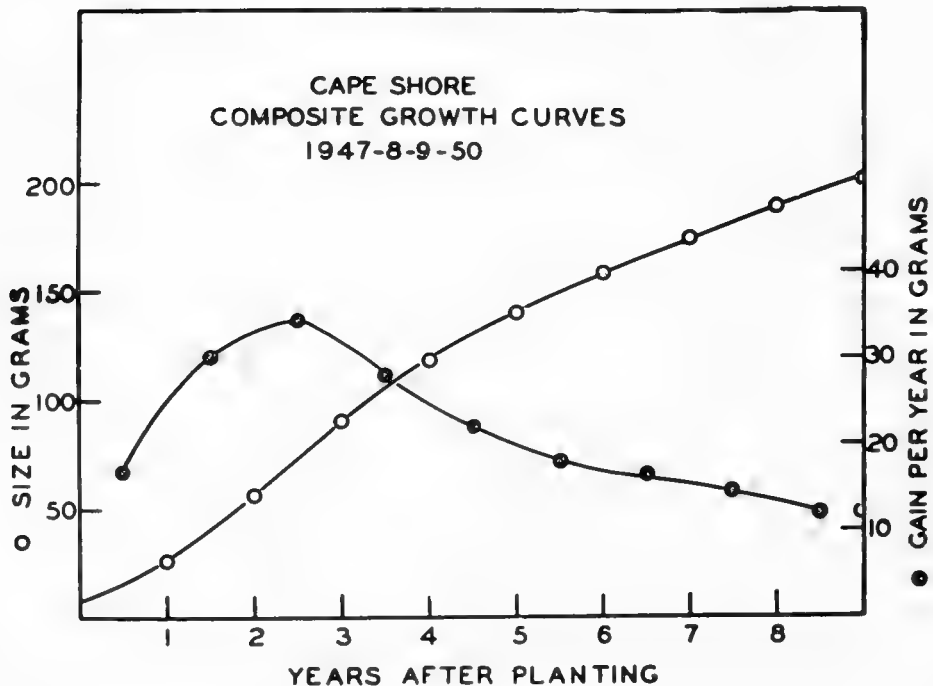

Figure 2.

The four years considered here cover a wide range of meteorlogical conditions from hot, dry summers in 1948 and 1949 to cool, wet summers in 1947 and 1950. Barring catastrophic changes, it is believed that the composite growth curve, based on data for these four seasons, will be modified only slightly by the accumulation of more growth data.

The second curve in figure 2 (solid circles) is particularly valuable in considering commercial applications of the growth data. The open circle curve like all those in figure 1 is a cumulative curve since every point indicates the accumulated weight of the clam up to the time indicated. The solid circle curve is an absolute growth curve derived from the cumulative growth curve. Each point plotted indicates the average gain in weight per clam per season. This curve shows that the 10-gram seed made the greatest absolute gain in weight (34 grams) in the third season after planting. After this peak growth the absolute gain per season drops off progressively until the sixth season when it levels off at 12-16 grams.

Figure 3 summarizes the essential growth data for the Cape Shore. In terms of volume yield from seed clams the clam farmer would be ill-advised to harvest any of his crop prior to the end of the third season after planting. At this time, assuming no mortality, from each bushel of seed he would have 9 bushels of "cherrystones" averaging 90 grams at about 400 to the bushel. Also in terms of volume yield he should probably harvest no later than the end of the sixth season when his growth rates have levelled off. At this time he would have 16 bushels of small-to-average "mediums" averaging 160 grams at about 225 to the bushel.

CALCULATED
GROWTH CURVES
1950

CAPE SHORE
SPERMACETI COVE
RARITAN BAY-EDGE COVE
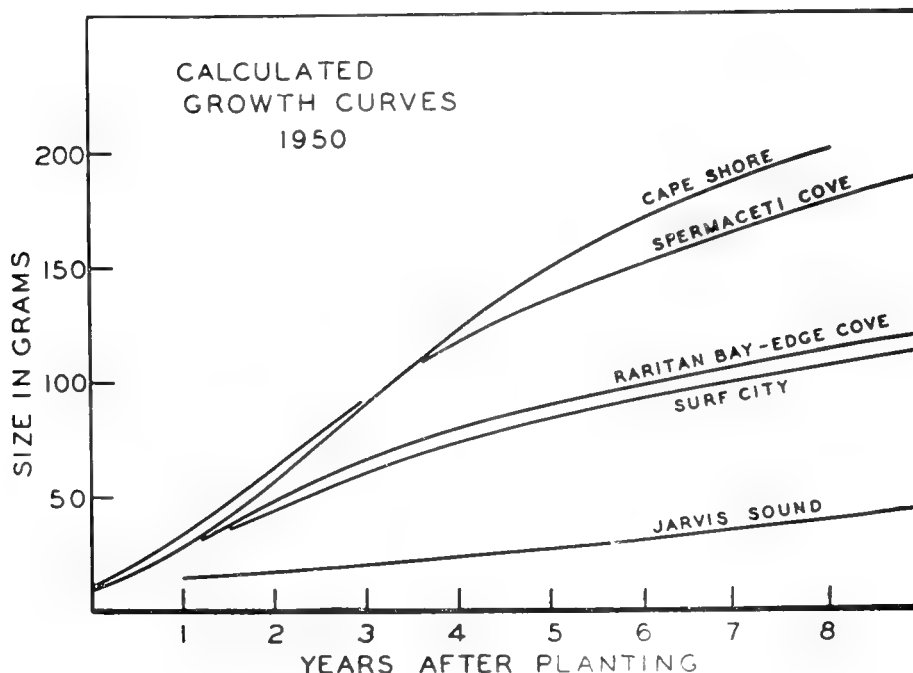SURF CITY
JARVIS SOUND

SIZE IN GRAMS

YEARS AFTER PLANTING

Figure 3.

The report in 1949 showed the growth curves for Jarvis Sound, Surf City, Edge Cove and Raritan Bay for 1948 in comparison with the 1947 and 1948 curves for the Cape Shore. It pointed out that in 1948 the best growth occurred in Jarvis Sound and the poorest in Raritan Bay. Surf City and Edge Cove had identical growth rates which were intermediate between the best and the poorest. In 1949 Cape Shore growth was best, Jarvis Sound had declined sharply, Raritan Bay had improved and growth at Edge Cove and Surf City was practically unchanged.

Cumulative growth curves for 1950 are shown in figure 3. Various points concerning them are listed:

a. Cape Shore growth is again best.

b. Second only to Cape Shore growth is that observed at Spermaceti Cove, Sandy Hook, a location studied intensively for the first time in 1950.

c. Growth at Edge Cove is again comparable to that at Surf City and is practically unchanged since 1948.

d. There are two striking contrasts with the curves for 1948. Growth at Jarvis Sound has declined from the best to the poorest of all locations. Growth at Raritan Bay has improved from the poorest to equal that found at Edge Cove. Probable reasons for these sharp changes in growth rates at the same location in successive years will not be discussed here.
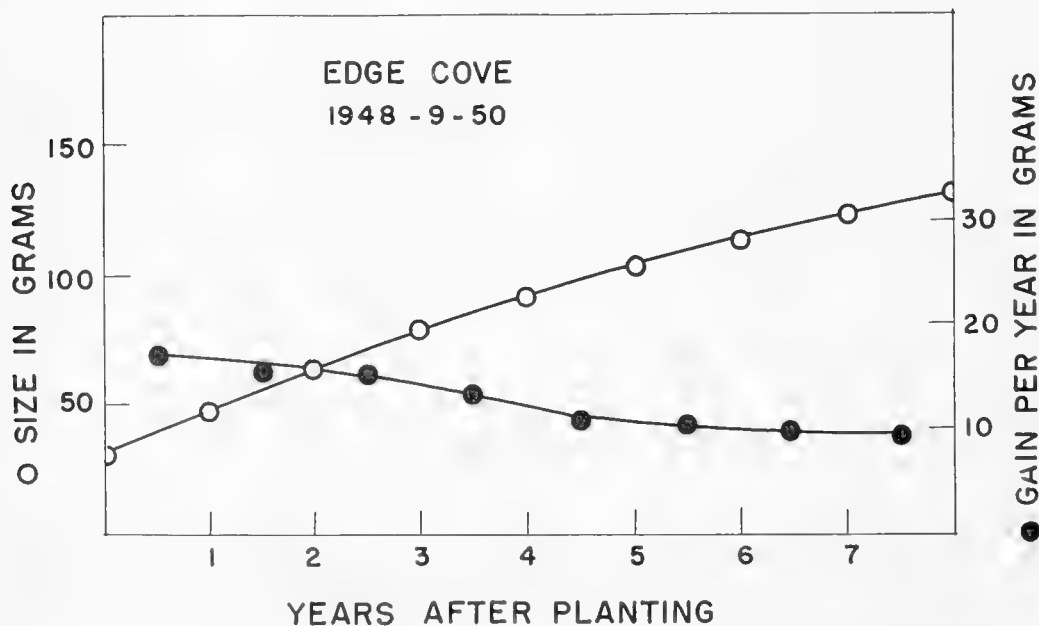

Figure 4.

Figure #4 presents cumulative and absolute growth curves for Edge
Cove based on the three growing seasons of 1948-50 inclusive. Unfortunately
data for clams smaller than 30 grams are not available for this station.
It is apparent, however, that the absolute growth picture differs markedly
from that obtained at the Cape Shore. Here the greatest gain per season
is obtained in the season when the 30 gram seed are planted. The gain
per season levels off after six growing seasons when the clams are still
only at "cherrystone" size. It seems obvious that a clam planter in this
area should not attempt to produce larger market sizes. This area is of
particular interest since it is one of the coves of Little Egg Harbor
which produces a large proportion of the hard-clams sold from New Jersey
waters and which has a tremendous population of hard-clams. In corrobora-
tion of the slow growths shown by our experimental plantings, examination
of a shipment of 100 bushels of "mediums" from Little Egg Harbor indicated
that they were approximately 15 to 20 years old.

In the course of our work a limited amount of data on the nutrients
available to the clams in the various experimental location has been ob-
tained. For the summer of 1950 Mr. Lawrence Pomeroy, one of our graduate
students, employed by the U. S. Fish and Wildlife Service made extensive
nutrient studies in the Edge Cove Area. Less extensive studies for this
summer were made on samples from Spermaceti Cove, Jarvis Sound and the Cape
Shore. One series of analyses for all locations during the month of August
1950 is presented below. In comparison with other analyses made at the
Cape Shore the figures given here are low. The average total phosphorus
and chlorophylls at this station are about 50% higher. The Edge Cove
results for August 1 are the peak values obtained for the 1950 season.
Those given for August 16 are near the season mean. The total phosphorus
level is an index to the total amount of organic material that may be pro-
duced in the area while the particulate phosphorus and chlorophyll are an
index to the organic materials present at the time of the analysis.

TABLE I

*Nutrient Data August 1950

Location	Date	Total P	Inorganic P	Parti-culate P	Total Diss. P.	Chloro-phyll
Sperm. Cove	Aug. 22	7.8	2.5	2.2	5.6	0.056
Cape Shore	Aug. 15	4.0	1.4	2.0	2.0	0.028
Edge Cove	Aug. 1	3.5	2.4	1.3	2.2	0.012
Edge Cove	Aug. 16	2.7	2.1	0.6	2.1	0.006
Jarvis Sound	Aug. 17	2.1	1.1	.46	1.6	0.005

* All phosphorus concentrations are given in microgram atoms per liter of
  sea water. Chlorophyll concentrations are given in milligrams per liter.

The locations in this table are listed in order of decreasing total
phosphorus present and in order of decreasing chlorophyll. This order
closely approximates the order of decreasing growth rates as shown in Figure
3. It appears that in areas of poor growth, the food supply is limiting.

Table I raises the question. "Why should there be such great differences

in food levels in these various locations?" The answer to this is not completely known but certain features may be indicated. The plantings at the Cape Shore and in Spermaceti Cove are swept by strong tidal currents and are probably favored by receiving the nutrients discharged from highly polluted rivers into large volumes of salt water. In contrast, the plantings at Surf City and Edge Cove are in partially stagnant water, oscillating between Barnegat Inlet and Beach Haven Inlet. No significant quantities of land drainage reach these locations and there is no through-circulation of tidal currents. The food shortage is probably intensified by the huge population of grazers (hard-clams) in Little Egg Harbor. The plantings in Jarvis Sound receive the full sweep of tidal currents but there is no evidence of significant land drainage into the Sound.

In consideration of the environmental factors most favorable to growth and "fattening" of hard-clams, it seems highly significant that our best growth rates have been obtained in areas with sparse native populations. Conversely, the areas supporting large native populations are showing only mediocre growth rates. There are various possible interpretations of this observation. For example, competition for food by the native population may be critical. It appears highly probable, however, that conditions which facilitate the setting of larvae, and thus the maintenance of the large population, are unfavorable for rapid growth. The effects of extensive land drainage and through-circulation on growth rates, have been indicated. Such a circulation with rapid "flushing" is not favorable for the conservation within it of a pelagic larval population unless the larvae are "hugging" the bottom. We may have here the reason why Little Egg Harbor with its oscillating, semi-stagnant water mass has a large, self-sustaining population of hard clams. The larval broods are not swept out of the area by strong currents, though there may be a high mortality due to predators, starvation, storms, etc. On the other hand, setting of clam larvae in locations swept by strong tidal currents, such as the Cape Shore flats, would be fortuitous, depending on the transportation of larvae to the area at the time of their maturity. The occasional reports of intense sets in such locations would be consistent with this idea.

It follows that one may expect to obtain the best growth rates by planting seed quahaugs in areas which may not necessarily be supporting large native populations. It has been proposed that growth rates may also be improved by using selected, fast-growing stocks obtained through a system of controlled artificial propagation such as that developed by Dr. Victor Loosanoff and his co-workers at the Milford Laboratory. Due to the hydrographic considerations discussed above, it is not expected that such stocks would establish themselves and be self-propagating in the areas where best growth rates are obtained. Thus the maintenance of optimum growth rates with selected stocks in such locations would involve a program of sustained artificial propagation or of transplantation of selected seed from other areas.

To summarize the principal points of this paper it may be stated that:

a. One season's data are inadequate in predicting the growth rates for a given location.

b. A good correlation between growth rate and available food materials is indicated.

c. Those areas in which the best growth rates are obtained support only a sparse native population and conversely areas in which there are extensive native populations show poor growth rates. It is proposed that this observation is correlated with land drainage and the circulation pattern.

SEASONAL GROWTH OF OYSTERS (C. VIRGINICA) IN FLORIDA

Philip A. Butler, Fishery Research Biologist
USFWS Pensacola, Florida

The pattern of growth in the commercial oyster is of fundamental importance to the grower because of its bearing on meat production. Environments noted for rapid increases in shell length, for example, may be of inferior value commercially unless such length increases are accompanied by corresponding volume increases. Some of the environmental factors known to affect growth patterns are: population density, type of cultch and substratum, density of commensal organisms, food supply, temperature and salinity. Despite the variety and complex interactions of these several factors, typical growth patterns are usually quite well defined in local environments. Knowledge of these patterns is helpful to the biologist as well as to the commercial producer.

The material presented here summarizes observations on the growth of individually identified oysters at Pensacola during the past three and a half years. Data are based only on oysters surviving at the end of the experimental periods indicated. In only one instance, 3-year old oysters, are there less than 50 specimens in the group; in others, the number of individuals varied from 60 to 200. Variations in the amount of growth in successive years and in adjacent but slightly dissimilar environments may obscure the basic pattern. Annual increases may vary, too, because of individual qualities in the oysters. For these reasons, all data are presented as percentage increases during stated time intervals; total annual increase is considered as 100 per cent.

MATURE OYSTERS

Growth patterns change as oysters become older. In this area, oysters may reach marketable size in 18 to 24 months and the pattern of growth during this, the second, year is described here as typical of the mature oyster. These data, Table 1, summarize the results of several experimental groups in three successive years. Variations in the timing of growth increases may be expected in years having warmer or colder than average winters.

TABLE 1

Per cent Increases by Month in Mature Oysters

	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Volume	10	5	6	6	10	12	10	9	12	6	7	7
Weight	10	7	8	3	9	9	10	12	11	8	7	6
Length	8	4	7	1	12	11	12	18	11	5	7	4
Width	7	4	8	1	4	13	15	28	8	7	4	1

The relative uniformity of monthly increases in volume and weight contrast notable with the seasonal pattern of changes in length and especially width

(dorso-ventral axis). Although the winter months of November-February are the best growing months, significant increases in all dimensions occur in August when water temperatures are typically highest ($34^\circ C$.). September, the poorest grow-ing month of the year, is characterized by the final wave of spawning. Oys-ters in the early part of this month are typically in their most debilitated con-dition. Poor growth rates in May can also be correlated with the reproductive cycle which at this time is at its peak of catabolic activity. This apparent slowing down of growth in conjunction with stages in the reproductive cycle con-trasts with conditions in northern waters where the two processes do not appear to be antagonistic.

IMMATURE OYSTERS

The growth rate of oysters during their first year is characterized, as might be expected, by its rapidity and also its uniformity. Analyses of monthly percentage gains in length, width, weight and volume show only minor fluctuations from month to month, and a tendency for a gradual decline in rate of growth with increasing age. Percentage increases during this first year show little similar-ity to the seasonal pattern of growth established during the second year. Table 2 shows monthly percentage gains in the growth of first year oysters.

TABLE 2

Per Cent Increases by Month in Immature Oysters

	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Volume			13*	8	9	10	6	6	12	7	11	13
Weight			16*	9	7	9	7	10	11	9	12	10
Length	14	15	10	9	11	8	6	8	7	5	3	4
Width	16	14	9	9	8	8	9	8	8	4	4	3

* Initial measurements of volume and weight were made at two months of age, so these data include increases for first three months.

In Table 3, monthly percentage increases in width are contrasted in yearling and mature oysters. Similar comparison of length, weight and volume illustrate the relative absence of seasonality in growth rates of young oysters. This situation is quite different from northern waters where low temperatures induce hibernation and growth is suspended during two to five winter months. For this reason, southern oysters enter their second season with a decided growth advantage.

TABLE 3

Per cent Increases in Width during First and Second Years

	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Immature	16	14	9	9	8	8	9	8	8	4	4	3
Mature	7	4	8	1	4	13	15	28	8	7	4	1

FACTORS AFFECTING GROWTH PATTERN

I should like to illustrate two of the many factors which may alter growth patterns in the same or in different environments.

The effect of aging on the growth pattern is quite dramatic, as may be seen in Table 4.

TABLE 4

Per Cent Increases in Length by Month and Year

	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
1st year	15	12	8	11	12	10	6	8	6	5	4	3
2nd year	3	6	5		3	11	7	31	25	4	2	3
3rd year		11			3	17	20	28	17		4	

Monthly increases become less and less uniform as the oyster ages and during the third year, 85% of the annual growth in length occurs during the four winter months. These data are based on only 37 surviving oysters and hence differ somewhat from data given in Tables 1 and 2, where larger populations were observed.

The effect of temperature on the timing of growth increases is well shown by data on volume changes in mature oysters grown at Pensacola contrasted with oysters observed by Dr. Loosanoff in Long Island Sound. It should be noted, Table 5, how the hibernation period extending from November through March telescopes the major annual volume increase into a few months. Volume increases rise gradually to a peak in August and rapidly decline with the onset of cold weather. At Pensacola, annual increases in volume are fairly well distributed throughout the twelve months.

TABLE 5

Per Cent Increase by Month in Volume - Mature Oysters

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Long Island Sound				4	3	9	13	30	24	11	6	
Pensacola	9	12	6	7	7	10	5	6	6	10	12	10

INHERITANCE OF GROWTH PATTERNS

The importance of the local environment rather than heredity in controlling growth patterns is illustrated by data on oysters which set in Chesapeake Bay and were transferred to Pensacola when six months old. When these oysters were one year old, i.e. after six months acclimatization, they were paired off with local oysters of the same age. In the following twelve months, percentage increases in length, width and weight of both imported and local oysters were

practically identical. However, real increases were significantly greater in the local stock. It is of interest that the smallest increases were obtained during the months of May and September, as expected. In the Chesapeake Bay area, where these oysters originated, September and May are characterized as especially good growing periods.

DISCUSSION

These observations on the growth of oysters at Pensacola indicate the complexity of studies on this problem. Variations in growth rates due to temperature, age, source of oysters, etc., may interfere with the correct interpretation of data. It is important to conduct experiments on oysters of known background and during comparable periods of time. The use of mixed year-classes in a single experiment may invalidate conclusions drawn from averaged data. Because of differences due to environment and to heredity, similarity in size may be a very poor criterion for selecting oysters of similar age. I have had oysters raised continuously in the same tray and known to be within twelve hours of each other in age which, at three years, differed in total size by 300 per cent. Conversely, oysters from some environments may be surpassed occasionally in overall size by the succeeding year class. Caution must be used too, in applying experimental results to areas having intense spatfalls. Oysters selected from such environments are inevitably the fastest growers since survival is primarily a matter of over-growing one's neighbor in such areas. Similarly, when spat are selected in very early stages and separated for experimental purposes, there is an artificial interference with the laws of survival. Slow growing oysters which could not have persisted in the natural environment may flourish in experimental trays to the detriment of the interpretation of results.

It is equally important that all possible phases of growth should be studied concurrently. If length and width measurements alone were recorded on mature oysters, it would be reasonable to assume that, in Pensacola for example, there was essentially no growth during May and September. However, when changes in weight and volume are recorded too, it is found that substantial increases do occur in these months.

There appears to be a fundamental difference in growth processes involving weight and volume as compared to length and width. Increases in length and width after the first year are discontinuous, frequently regressive, and show a gradual decline on a percentage basis from the first month of an oyster's life until its death. Increases in wieght and volume are quite the reverse. During the first three years at least, they show a gradual increase in annual increment. It is important also to distinguish between growth of shell and growth of meat, since these two are not necessarily concomitant. Mortality data obtained in these experiments but not reported here, suggest that the total life span of oysters in southern waters may be appreciably less than in the colder waters of the north.

AN OYSTER FEEDING EXPERIMENT

Willis G. Hewatt

Texas Christian University, Virginia Fisheries Laboratory
and Texas A. & M. Research Foundation

The subject of "oyster food" had been one of the foremost problems of the oyster biologist during the present century. Notable contributions have been made recently in this country by Thurlow Nelson (1925), and George MacGinitie (1941), Victor Loosanoff (1947), and the work reported at this meeting by Dan Floyd and Harry Davis, and James Lackey. In Europe the subject has been studied by R. E. Savage (1925), C. M. Yonge (1926), R. Sparck (1925), and P. Korringa (1949).

There is still no general agreement upon the types of organisms and the sizes of food particles which can be utilized by the oyster.

In an effort to add information to our knowledge of oyster food, a series of observations was conducted on three groups of Louisiana oysters over a period of six months in 1949. Three groups of oysters, consisting of 14 oysters in each group, were maintained under different environmental conditions over the time period.

The "Experimental Group" of oysters were placed in numbered finger bowls on a water table. Each bowl received 50 liters of water per hour. The water supply for this group of oysters passed through a salinity-turbidity recording instrument, then through a baffled settling tank and finally was filtered through a monel metal filter cloth of 200 mesh per linear inch. All organisms above 50-60 microns were filtered from the water supplied to the experimental oysters.

Another group of 14 oysters, which we shall call the Control Group, were also placed in finger bowls but each one was supplied with unfiltered water.

A third group, "Master Controls" were placed in numbered compartments of a wire tray, which were permitted to rest on the sandy-mud bottom of Barataria Bay, Louisiana, where the experiments were conducted.

At the end of each week over a period of six months each of the oysters of the three groups was weighed under water. A triple-beam balance was employed in the weighing procedure.

At the end of the experiment a glycogen analysis was made on each surviving oyster.

The following results were obtained from the experiment:

1. The mortality records on the relatively small group of oysters conformed very closely to the tray mortality records taken from large groups of oysters in the same area.

2. The mean glycogen content of the ten surviving Experimental oysters was 0.55 per cent. For the six Control oysters the percentage was 1.55 and for the Master Control group it was 1.13.

3. At the end of the period of approximately six months the Experimental oysters had gained only 19 per cent of their original combined weights. The Control oysters had increased 30 per cent, and the group which hung from the piling in a tray had increased 39 per cent.

4. Similar experiments have been conducted at the Virginia Fisheries Laboratory and the results were very similar. The method of weighing an oyster under water at intervals, appears to give a dependable record of the condition of the oyster without sacrificing it. In most instances the death of an oyster can be predicted several weeks before it becomes a gaper.

Preliminary Report on the Chincoteague Bay Survey

Fred W. Sieling and J. W. McGary

An ecological survey of Chincoteague Bay was started in August 1951 to study the cause or causes of the decline of the seafood industry in the region and to explore means for the rehabilitation of the Bay. The area to be studied runs from Fenwick Island Light to Fishing Point, Virginia, which includes Chincoteague Bay, Sinepuxent Bay, Isle of Wight Bay, Asawoman Bay and Little Asawoman Bay. This whole area is a continuous body of water which is served by two inlets which are located at Ocean City, Maryland, and Chincoteague, Virginia. The boundaries between the areas are artificial rather than actual and a system of interlocking tides and currents make it impossible to treat them as separate areas.

The Chincoteague Bay area consists of a shallow lagoon separated from the Atlantic Ocean by a narrow wave-built bar which averages about one-quarter of a mile in width. The total length of the area is about 40 miles and the average width is about three miles, though it varies from $\frac{1}{4}$ mile to 5 miles. The depth varies from 20 to 40 feet at the inlets to a maximum of 8 feet in the channel thru the Bay, while most of the area has a depth of from 2-4 feet. The water shed of the area is small being only about 205 sq. miles, and the total water surface of the bay at mean low tide is approximately 120 sq. miles.

Upon examination of the old Coast and Geodetic Charts and the army Engineers overlays, it was found that four major changes have occurred since 1847, namely the closing of Green Run Inlet, the opening and subsequent closing of an inlet three miles south of Ocean City, the opening of the present Ocean City Inlet, and the build-up of the hook at the southern end of Fishing Point.

The only change that can be noted along the off shore side of the bar is a general smoothing of the shore. There is no evidence of a shoreward migration of the bar since 1947. On the inshore side of the bar there is a broad shoal area on the eastern side of the bay. The barren nature of the bottom and the laminated nature of the cores taken from the bottom indicates a possible filling of the bay by sanding due to wind transport or by breakers coming over the beach during intense storms. The western side of the bay is characterized by low marshes around the mouths of small streams which discharge into the bay. These marshes are most extensive in the central part of the bay and it could not be determined to what extent the marshes had built out in the past hundred years, but there is evidence that the low islands in the west central part of the bay are eroding rather rapidly.

Salinity patterns by season are plotted and shown by slides. The salinity of the bay varies as is to be expected with the season of the year. During the summer it is quite high, being at times higher than the ocean salinity, due to the high evaporation rate, the small watershed, and the shallowness of the bay. The salinity in the spring and winter is lower in general than that of the ocean due to the low evaporation rate then and the increased rainfall. Salinities commonly have values as high as 35 parts per thousand. This salinity fluctuates rather quickly as the bay is shallow and a heavy rainfall will depress the salinity sharply but the presence of the

inlet at each end with its reservoir of high saline water quickly brings the water back to its normally high salt content. The salinity is at present being studied thru a series of monthly surveys which follow a predetermined pattern. The sampling stations are laid out in a series of parallel transverse sections which cover the bay from Virginia to Delaware. This coverage gives a rather complete picture of the salinity pattern each month and in addition to this, daily salinity samples are taken at the laboratory at Public Landing.

On these sampling trips temperature, oxygen, and turbidity are also included in the data taken. It has been found that the temperature fluctuates rapidly, responding to the climatic conditions. During the summer, water temperatures are taken at the lab by means of a recording thermograph in addition to the monthly sampling trips. Vertical gradients are found in the deeper parts of the bay and are occassionally as great as $1.5^{o}C$. Horizontal gradients are much greater and occur toward the inlets where the influence of the ocean water is felt to a greater degree.

Dissolved oxygen was determined at all bottom stations on each of the monthly sampling trips. The average value for dissolved oxygen for each of the sampling trips showed a slightly increasing deficiency from September until the April trip when the deficiency began to decline. A negative result was obtained when an attempt was made to correlate this deficiency with the other variables measured, namely salinity, water temperature, depth, turbidity, and wind velocity. Further examination showed that one of the geographical divisions of the bay, namely Sinepuxent Bay, showed a greater deficiency than the other areas. No explanation is offered for this.

Throughout the area the tidal range is not great, being of course greatest at the inlets and the least at the center of the bay. At the inlets the greatest tidal difference was found to be 5.8 feet, while at the center of the area the greatest difference was found 1.5 feet. The normal tidal amplitude at the inlets is about 4.5 feet and at the center is only about 0.5 feet.

Currents are being measured throughout the bay in an effort to determine the movement of the water in the area and the velocity of these movements. The wind effects are marked and produce a wind circulation pattern. The slight currents present in the bay, being only about 0.3 knots, bring difficulties in measurement which are not yet overcome. At present a version of the chip log is being used to measure the currents.

Turbidity is being measured but there seems to be no correlation except with wind as the water is shallow and easily roiled and the bottom muddy. The turbidity is being determined with a Fisher Electrophotometer using several wave lengths of light to see if there is any difference in the size of particles of suspended matter. We are using standard filters of 425,525, and 650 Angstrom units. The bay clears very quickly after being muddy, however.

Bottom cores are being taken and examined by geologists to note the rate of filling of the bay and also to see to what extent sanding from seas coming over the beach has progressed. It is hoped to compare bottom cores from good and bad oyster bottom in the area.

In addition to the hydrographic studies just outlined, the biological part of the survey is proceeding along several lines. As one of the main reasons for the initiation of this program is the decline in the oyster production in the area, a large part of the investigation deals with oysters. Very little scientific information is available for the region and so a large part of the work is the collection of more or less basic information.

A year round study is being made of the attachment of many of the fouling organisms by using shell collectors. The period of attachment of many of these organisms is unknown for this area and of course is important for the commercial oyster production in the bay. Along with this a study of the setting of the oyster spat is also being made in different areas of the bay as the distribution of set is not known for Chincoteague Bay. In the past there has been no attempt to plant shells in the Maryland part of the bay and to obtain seed oysters. Because of this policy in the past the most efficient time to plant shells in the area is unknown. As part of this program we have planted on some of the old natural oyster rocks and in a new area several hundred bushels of brood stock oysters to be spawners. This was done in March and then in June and July the areas were planted with about 10,000 bushels of shells. The plantings on the old natural rocks have to date received a light set of oysters but the new area which is designed to be a seed area has not to date. The old rocks were totally barren of oysters but the new area had a natural growth of oysters. Last summer's setting records show no set for the old oyster bars but did show a rather heavy but late set in the proposed seed area. The areas planted were selected on the basis of last year's setting records and information from watermen.

Growth of oysters from various areas planted in Chincoteague Bay is being studied in order to determine the best source of seed for the planters operating there. In addition to this oysters from several areas are being held in trays in different parts of the bay to determine mortality under different conditions, both submerged and intertidal. As yet no conclusive data are available on these experiments.

The predator problem is being studied also in relation to the oyster drills, in an effort to develop controls that can be used locally. These pests are a serious problem in the area and the local planters do nothing to control them except to move the oysters. Parasites of oysters in the area are to be studied but as yet have not been worked on in a systematic manner.

It is planned also to devote time to the experimental growth of oysters on trays on a semi-commercial basis as this may be an answer to the predator problem in the bay and also eliminate some of the loss due to the soft texture of the bottom.

This summer a survey of the population of clams (Venus) in the area is being made to try to get an idea of the population density and the a   composition of the animals in the area. The clam here is Venus mercenaria and at present is caught on a large scale commercially. However, some in the area fear depletion and as usual others claim they are here to stay. We propose to have figures and then several years from now repeat the sampling stations and so have a comparison of the population at that time. No live

specimens of Mya have been found in the bay as yet but many dead shells.
Arca and Ensis are present in numbers rather widely distributed in the areas.
Attention is being paid to the different forms found associated with the clams
in the different depths and parts of the bay as part of the ecological study
of the area.

This summer a survey of the protozoa of the area is being made as part
of the overall project. This is to be continued for several summers in an
attempt to classify the forms found in the bay. This work is being done by
Dr. McDowell.

A checklist of the forms found in the area is to be worked up as part
of the project. Collections are being made and will be identified as soon
as possible.

Several other minor investigations are under way or proposed and will
be part of the overall program as it progresses. I hope to give you a more
complete report at a later meeting.